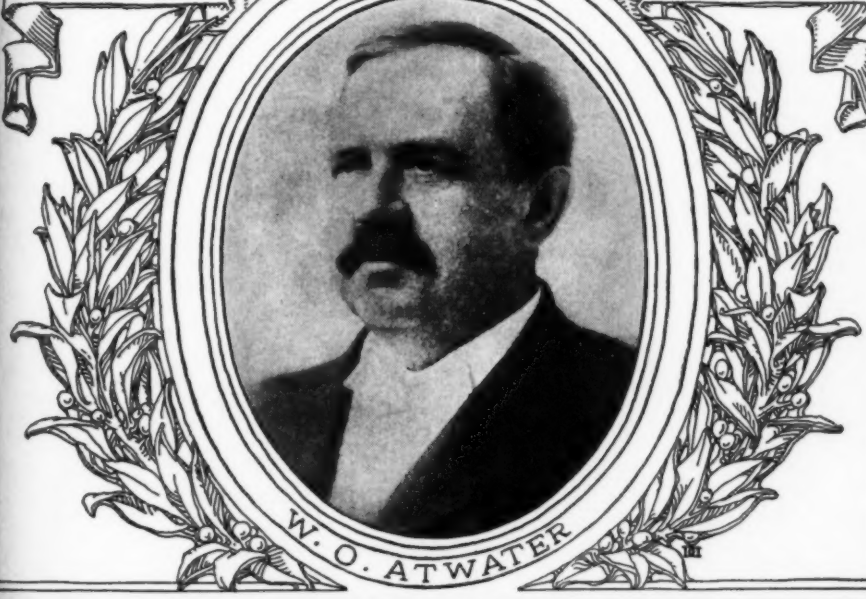
MARCH 10, 1944

THE JOURNAL OF NUTRITION

VOLUME 27 NUMBER 3



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Official Organ of the American Institute of Nutrition
PUBLISHED MONTHLY BY
THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
PHILADELPHIA 4, PA.
Price, $5.00 per volume, Domestic; $5.50 per volume, Foreign

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NUTRITIONAL REQUIREMENTS OF THE SYRIAN HAMSTER

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(Received for publication July 22, 1943)

Routh and Houchin ('42), in a preliminary report, stated that hamsters require thiamine, riboflavin, pyridoxine, pantothenic acid, and nicotinic acid. More recently Cooperman, Waisman, and Elvehjem ('43) have described a study of the requirement of these animals for the water soluble vitamins. The hamsters grew at a normal rate when the diet was supplemented with nine members of the vitamin B complex, including thiamine, riboflavin, calcium pantothenate, pyridoxine, nicotinic acid, sodium para-aminobenzoate, inositol, choline, and biotin. A few females were carried through reproduction with fair success. The authors concluded that the hamster does not require nicotinic acid. They were uncertain whether both inositol and para-aminobenzoic acid are required, but they were convinced that at least one of these is required. Our observations on the nutritional requirements of these animals are described in the following pages.

EXPERIMENTAL

Since many investigators are not familiar with the habits of hamsters [2] a brief description of the routine management of our colony is included. Ordinarily the animals are quartered in square cages of $\frac{1}{2}$ inch mesh hardware cloth, with floors of this same material. The food is supplied ad libitum in glass jars, and water is provided from inverted bottles with glass drinking tubes. Hamsters are usually gentle and easily handled, though on rare occasions a female has become vicious. As the animals approach sexual maturity the males and females are segregated, with three or four together to a cage. The females, especially when pregnant, are much more pugnacious than the males and if caged together the males may be badly scarred. For convenience the breeding animals are usually confined in individual cages.

[1] Contributions from Missouri Agricultural Experiment Station, journal series no. 911.
[2] Our foundation stock was purchased from Henry Bergman, Springfield, Mo.

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When the females are 8 or 9 weeks of age they are mated to a male not closely related and at least 12 weeks of age. The females are placed in the cage with the male and are removed after 5 minutes if not receptive and after 10 or 15 minutes if receptive. On the thirteenth day of gestation the floors of the cages are removed and a small quantity of shredded paper is provided for a nest. The females are disturbed as little as possible on the sixteenth day and for the first 2 days following birth of the litter. If a female is disturbed shortly after parturition she almost invariably becomes excited and kills and eats some of her litter. The young are not handled until 1 week of age, and are weaned when 21 days of age. The females are given a 2-week rest period between the weaning of a litter and remating, and are retained until approximately 1 year of age when their usefulness declines. Female hamsters attain a mature weight of 105–135 gm. while the males are somewhat smaller, weighing between 95 and 120 gm.

The composition of the stock ration, no. 1, is shown in table 1. Each female, during lactation, receives in addition 10 ml. of whole milk

TABLE 1

Composition of diets.

Ration	%		%
1 Ground wheat	28	Ground corn	20
Wheat germ	7	Dried yeast	10
Alfalfa meal	5	Calcium carbonate	1
Dried skimmilk	15	Sodium chloride	1
Linseed oil meal	12	Vitamin A-D mixture [1]	1
Basal mixture	%	*Vitamins: mg. per 100 gm. of basal mixture*	
2 Casein (acid washed and alcohol extracted)	20	Thiamine hydrochloride	0.8
Cerelose	65	Riboflavin	1.6
Lard	7	Pyridoxine hydrochloride	1.2
Cellulose	3	Calcium pantothenate	1.0
Salt mixture [2]	4	Nicotinic acid	5.0
Vitamin A-D mixture [1]	1	p-Aminobenzoic acid	100.0
		Inositol	250.0
		Choline chloride	400.0
		2-Methyl-1, 4-naphthoquinone	3.0
		Alpha tocopherol	2.5

[1] Composed of a mixture of 2 parts Mead-Johnson oleum percomorphum and 98 parts of lard. One gram of the mixture supplies 1200 I.U. of vitamin A and 170 I.U. of vitamin D.

[2] A modification of mixture 351 of Hubbell, Mendel, and Wakeman ('37), in order to increase the phosphorus content:

$CaCO_3$	125.2	KH_2PO_4	212.0
$Ca_3(PO_4)_2$	376.3	$FePO_4 \cdot 4H_2O$	20.5
$MgCO_3$	25.0	$MnSO_4 \cdot 4H_2O$	25.5
$MgSO_4 \cdot 7H_2O$	32.8	$CuSO_4 \cdot 5H_2O$	1.4
NaCl	69.0	$Al_2(SO_4)_3K_2SO_4 \cdot 24H_2O$	0.17
KCl	112.0	KI	0.08

daily. Several females have borne and reared five litters while consuming the stock ration, and in no cases were nutritional deficiencies observed. The heaviest mortality takes place during the first 5 days of life and is especially heavy during the first 2 days. The fact that the young are not counted until the second day after birth accounts for the relatively small litter size.

GROWTH ON SIMPLIFIED RATIONS

Adequacy of synthetic vitamins [3]

The first step was to determine whether a simplified ration containing only the known vitamins is nutritionally adequate for growth. The first ration studied is very similar to the one that was shown by Richardson, Hogan, Long, and Itschner ('41) to be adequate for growth of the albino rat. The composition of diet no. 2 is given in table 1. The simplest experimental diet contained the six vitamins, A, D, thiamine, riboflavin, pyridoxine, and calcium pantothenate. For convenience the other vitamins included in the various diets are given in table 2 .

The amounts of the vitamins in the various diets, if included at all, were the same as in ration 2. No effort was made to determine the

TABLE 2

Vitamins included in rations.

RATION NUMBER	NICOTINIC ACID	CHOLINE	INOSITOL	p-AMINO-BENZOIC ACID	BIOTIN	VITAMIN E	VITAMIN K
Rations used for studies on growth							
2	+	+	+	+	..	+	+
3	−	+	+	+	..	+	+
4	−	−	−	−	..	+	+
5	+	+	+	+	..	−	+
6	−	−	−	−	..	−	+
7	+	+	+	+	..	+	−
8	−	−	−	−	..	+	−
9	−	−	−	−	..	−	−
Rations used for studies on reproduction							
2	+	+	+	+	−	..	..
10	+	+	+	+	+	..	..
11	+	+	+	−	+	..	..
12	+	+	+	−	−	..	..
13	+	−	+	−	−	..	..
14	−	+	+	−	−	..	..
15	−	−	+	−	−	..	..
16	+	+	−	+	+	..	..

[3] Supplied by Merck and Co., Rahway, New Jersey.

minimum requirement for any of them and in all probability the quantities supplied were larger than necessary. The average weekly weights of the animals on the various rations are shown in table 3. The rate of growth and the mature weights of animals that consumed ration 2 were equal to those of the stock animals. Hamsters grow at a normal rate on diets that contain no vitamins except those now recognized.

TABLE 3

Growth of hamsters on simplified diets.

Ration no.	COLONY DIET		SIMPLIFIED DIETS						
	1		2	2a	3	4	7	8	
No. of animals	12F	11M	5F	4M	5M	9M	10M	3F	8M
Age weeks	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
3	27	29	28	28	24	27	26	27	26
4	44	42	41	37	37	39	36	37	36
5	50	55	54	49	53	48	44	49	46
6	60	63	66	60	63	62	50	58	53
7	69	70	76	70	74	71	47	61	62
8	77	76	84	78	82	77	47	75	67
9	86	82	89	85	87	86	60	81	75
10	93	85	96	91	89	91	72	89	81
11	101	86	103	93	90	93	77	94	83
12	105	90	100	96	94	93	80	97	81
13	111	93	94	100	96	95	82	100	91
14	108	96	100	101	97	96	87	101	93
15	112	98	107	102	101	99	92	105	93
16	115	96	106	100	104	99	95	101	91
17	113	103	110	101	104	101	95	105	94
18	115	100	111	100	104	101	95	103	94
19	120	102	117	100	103	102	96	104	94
20	117	105	120	100	102	101	100	...	93
21	123	106	121	101	102	104	101	...	93
22	113	109	119	100	104	106	102	...	95
23	110	106	119	103	106	107	105	...	97
24	111	108	122	103	109	109	107	...	97

Nicotinic acid

Ration 2a contains Labco [4] vitamin-free casein but in all other respects is identical with ration 2. Ration 3, except for the omission of nicotinic acid, is identical with ration 2a. The animals which consumed these two rations grew at practically the same rate and no abnormalities were observed. They were indistinguishable from stock animals of the same age and it seems certain that under our experimental conditions hamsters do not require dietary nicotinic acid for growth.

[4] Purchased from the Casein Co. of America, New York, New York.

Choline, inositol and p-aminobenzoic acid

An attempt was then made to simplify the diet still further and ration 4 was prepared which contains the four fat-soluble vitamins A, D, alpha-tocopherol, and 2-methyl-1, 4-naphthoquinone, and the four water-soluble vitamins, thiamine, riboflavin, pyridoxine, and pantothenic acid. The constituents of the diet may contain other vitamins as contaminants, but biotin is the only one for which a search was made. It was estimated [5] that the casein contained 0.0150 μg. of biotin per gram and the cerelose contained 0.0042. These two components would supply 5.73 μg. of biotin per kilogram of ration. Since the chick requires several times this amount, one would expect ration 4 to be inadequate for growing hamsters if they require dietary biotin. However, the animals on this diet grew rapidly and reached a normal mature weight. It was concluded that it is unnecessary to include either choline, inositol, or para-aminobenzoic acid in the diet of hamsters during the stage of growth.

Alpha-tocopherol

Two vitamin E-free diets, nos. 5 and 6, were used to determine whether this vitamin is essential for the hamster, but the animals responded in the same manner to both rations. The growth rate was normal at first, then gradually declined. The animals rarely exceeded 85 gm. in weight and invariably lost weight before death. Of the twenty-eight animals on these rations all collapsed. Six were treated and recovered and the other twenty-two died. The animals exhibited no abnormal symptoms, other than slow gain, or loss in weight, until less than 2 hours before death. The first abnormal symptoms were unusual activity, lack of coordination, and variable activities such as jumping in the air, rolling over, biting themselves, running about the cage, copious flow of saliva, and violent reaction to external stimuli such as touch or noise. These manifestations increased in intensity and approximately 1 hour before death the animals collapsed, lost consciousness, and lay in a stupor. Seven of the animals in this final stage were each treated with 5 mg. of alpha-tocopherol. One died within 10 minutes after treatment and the other six made spectacular recoveries. They regained consciousness within 45 minutes, and within 1 hour they were able to move about the cage, and after 12 hours they were eating. At this point alpha-tocopherol was included in their diets and thereafter they gained rapidly and those that were retained long enough reached

[5] The assays were made by Mrs. Barbara Long in Dr. V. du Vigneaud's laboratory, Cornell Medical College, New York, New York.

normal mature weights. Autopsy of the animals that died did not disclose any gross abnormalities but no histological examinations were made. The depletion periods before collapse varied from 4 to 18 weeks, but eventually all animals were affected. There is every reason to suppose the hamster could be used conveniently for the biological assay of alpha-tocopherol.

Vitamin K

Diets 7 and 8 did not contain 2-methyl-1, 4-naphthoquinone, and they were used to study the requirement of the hamster for vitamin K. The weanling hamsters grew normally for 2 or 3 weeks on both rations and then the growth rate gradually declined. Somewhere between the third and seventh week all of them either gained slowly, ceased to gain, or lost up to 20% of their body weights. During this period of arrested growth they became thin and presented an unthrifty appearance. Three animals at this stage were sacrificed for examination. All three had a considerable number of small hemorrhagic areas in the muscle and subcutaneous tissues and in the lining of the abdominal cavity. Free blood was also found in the sinuses. These hemorrhages were not fatal but they did retard the rate of growth or even caused a temporary loss in body weight. Following the period of arrested growth the animals grew rapidly and attained normal mature weights at approximately the same age as did similar animals receiving rations that contained vitamin K. The resumption of growth is probably explained by the establishment of intestinal flora of a type that can synthesize vitamin K to prevent hemorrhages.

Vitamins E and K

Several weanling hamsters were placed on ration 9 which contained neither vitamin E nor vitamin K. The growth rate on ration 9 was retarded to about the same extent as on the E deficient rations previously described. Of a total of seventeen hamsters, male and female, which received ration 9, fourteen died after depletion periods that varied from 6 to 16 weeks. The symptoms preceding death were the same as on rations 5 and 6, but autopsy revealed the hemorrhages that are characteristic of a vitamin K deficiency. Three of the animals were found in the unconscious stage that precedes death and 5 mg of alpha-tocopherol were administered to each. All three made rapid recoveries.

Vitamins required for growth

Other data, which will not be described, agree with Routh and Houchin ('42) in showing that the hamster requires thiamine, ribo-

flavin, pyridoxine, and pantothenic acid. The requirement of this animal for vitamins A and D has not been investigated, but it is assumed for the present that both are required. Vitamin E is essential and vitamin K must also be supplied if a normal rate of growth is to be maintained.

REPRODUCTION

Colony ration

The litter records are summarized in table 4. It will be observed that each of 6 females on ration 1 reared four litters, and that three of them

TABLE 4

Litter records.

RATION AND LITTER NO.		NO. OF LITTERS	NO OF YOUNG PER LITTER	NO. OF LITTERS WEANED	NO. OF YOUNG WEANED PER LITTER	WEANING PERCENTAGE	AVERAGE WEANING WEIGHT	DAYS PER LITTER [1]	NO. OF FEMALES AT CLOSE OF EXPERIMENTAL PERIOD	DAYS FROM LAST LITTER TO CLOSE OF EXPERIMENTAL PERIOD
	I	10	4.4	8	2.9	52	25.0	78	..	..
	II	10	6.2	8	4.0	52	29.0	56	..	..
1.	III	9	6.1	7	4.7	60	26.7	55	..	..
	IV	6	4.7	5	3.4	61	28.0	57	..	..
	V	3	3.3	3	3.0	90	24.6	81	7	91
2.	I	3	4.0	3	2.0	50	23.5	41	3	57
	I	3	5.7	3	3.3	59	21.0	64	..	..
10.	II	1	6.0	1	5.0	83	31.2	54	..	..
	III	1	4.0	1	2.0	50	27.5	50	3	66
	I	9	3.4	5	2.8	45	21.8	32	..	..
11.	II	2	2.5	1	1.0	20	24.0	78	..	..
	III	1	2.0	1	1.0	50	21.0	64	8	79
	I	12	3.7	10	3.1	70	21.6	53	..	..
12.	II	5	4.2	5	3.8	90	25.3	88	..	..
	III	2	2.5	2	1.5	60	23.7	90	12	115
	I	9	3.8	3	1.0	9	23.3	47	..	..
13.	II	2	3.0	1	1.0	17	24.0	50	..	..
	III	1	8.0	0	0.0	0	...	51	7	114
14.	I	6	3.0	3	1.0	10	24.5	41	..	..
	II	1	2.0	1	2.0	100	22.0	20	4	94
15.	I	6	3.3	2	1.3	22	23.3	51	..	..
	II	3	2.3	1	1.0	14	10.0	46	6	109

[1] This column signifies the number of days that intervened between litters. Under our procedure, the minimum, except for first litters, is about 51 if each litter is weaned.

reared a fifth. The percentage of young weaned varied from 52 to 90%, and the average weaning weights, at 21 days, varied from 24.6 to 29.0 gm. This record is fairly satisfactory but in all probability it can be improved. A certain amount of time was required to become familiar with the habits of the animals and it is believed that the mortality rate has been reduced by providing a more suitable environment. Furthermore it is not certain that the colony ration is adequate for optimum nutrition.

Synthetic diets

None of the experimental diets contained less than the eight vitamins that were included in diet 4. For convenience the other vitamins included in the various diets are given in table 2.

The three females on ration 2 bore three litters, and weaned 50% of their young, but the weaning weights were lower than on the colony diet. None of the females bore a second litter within a reasonable time and they were changed to another diet. However, it was certain that hamsters could attain some degree of success in rearing litters on synthetic diets and the study was continued, with diets containing various combinations of the available vitamins.

Biotin and p-aminobenzoic acid

Three females received ration 10, which except for the addition of biotin is the same as ration 2. The amount of the vitamin [6] supplied was 3 μg. daily per animal. Two of the females weaned one litter each. The third female reared three litters, and both the percentage of young weaned and the weaning weights were approximately the same as in the breeding colony on the stock diet, no. 1. Apparently the animals became depleted of another vitamin, however, for additional litters could not be secured. The response of the one animal that bore three litters gives some indication that the hamster requires both biotin and p-aminobenzoic acid for reproduction, but it may be impossible to arrive at a final decision until all other essential vitamins become available.

Ration 10 was modified by the omission of p-aminobenzoic acid to make ration 11, and by the omission of both biotin and p-aminobenzoic acid to make ration 12. The females on these two rations were about as successful in rearing litters as were those on ration 10 except the weaning weights were lower.

[6] S.M.A. Corporation Conc. no. 200.

Choline and nicotinic acid

The rations used to study the requirement of the hamster for these two vitamins during the reproductive stage were modifications of ration 12. Ration 13 contains nicotinic acid but no choline. Ration 14 contains choline but no nicotinic acid. Ration 15 contains neither. The females on all three rations bore litters that were apparently normal, but the number of young per litter was unusually low. Apparently lactation was subnormal since most of the young died within 1 week, indicating that both nicotinic acid and choline are required by the hamster for successful lactation.

Inositol

The inositol-free diet used most extensively was no. 16 which, except for the deficiency in inositol, is identical with ration 10. The females on the inositol-free rations have borne twenty-five litters, but twenty of these were still-births and the embryos were bloody, shapeless masses when expelled, usually at full term. Several females died during or shortly after parturition, with one or more decomposed embryos in the uterus. There were fifteen living young in the other five litters. One of the young was weaned and another died when 20 days old. The others died, or were killed by their mothers, before they were 1 week old. We assume that the mothers which bore living young had not been sufficiently depleted of their inositol reserves.

Vitamins required for reproduction

Nearly all the females bore one litter, a few bore a second, and occasionally one would bear a third. It seems quite certain that all of the diets were inadequate for reproduction, and that the hamster requires at least one vitamin that has not been recognized as yet. When all the experimental diets are inadequate, the significance of differences between diets that contain different numbers of vitamins may be uncertain. With this reservation in mind, our data indicate that during the reproductive phase female hamsters should receive at least seven members of the vitamin B-complex. In addition to the four that are required for growth the diet should include inositol, nicotinic acid and choline. The evidence concerning biotin and p-aminobenzoic acid was insufficient to justify listing them as essential vitamins. There has been no opportunity as yet to determine whether the hamster requires vitamin B_c (Hogan and Parrott, '40; O'Dell and Hogan, '43; Pfiffner et al., '43).

DISCUSSION

The nutritional requirements of the hamster have not been studied extensively, but the discrepancies that have appeared deserve some comment. We agree with Cooperman and collaborators ('43) that the hamster does not require nicotinic acid for growth. According to Cooperman et al. this animal requires biotin for growth but we were unable to repeat this observation. In our experience the growth rate of hamsters is improved by including vitamin K in the diet, but Cooperman et al. reported that their animals grew at a normal rate on rations that did not contain this vitamin. However, our animals consistently grew more rapidly than did those described by Cooperman and co-workers. Their male hamsters gained 1.0 gm. and the females gained 1.4 gm. daily in a 28-day period. Our males made an average gain of 1.5 gm. and the females 1.7 gm. daily, from the twenty-first to the forty-ninth day of age. The Wisconsin group also reported that the hamster requires either p-aminobenzoic acid or inositol, or possibly both, during growth. We did not find that either of these substances increased the rate of growth, though in the absence of inositol our females seldom bore living young. Thus far, biotin and p-aminobenzoic acid have not markedly improved any of the hamster diets we have tried, but a final decision is reserved until the trials can be repeated under more favorable conditions. An explanation of the discrepancies between our observations and those of Cooperman and associates, may be found in the interrelations between p-aminobenzoic acid, inositol, biotin, and vitamin K, which determine the rate at which vitamins are synthesized in the intestinal tract. Schmidt and Büsing ('42) report that when chicks receive a diet deficient in vitamin K there is a reduction in the number of intestinal bacteria. Black, Overman, Elvehjem, and Link ('42) have shown that if rats consume a synthetic diet to which sulfaguanidine has been added, they grow slowly and develop hypoprothrombinemia, presumably due to the depressed synthesis of vitamin K by intestinal bacteria. In a similar manner, a deficiency of biotin (Daft, Ashburn and Sebrell, '42; Martin, '42; Neumann, Krider and Day, '43; Nielsen and Elvehjem, '42) and of folic acid (Nielsen and Elvehjem, '42; Martin, '42) may be induced by the administration of sulfa drugs. One might speculate that when vitamin K is included in a diet such as no. 4, the synthesis of biotin, inositol, vitamin B_c, or other vitamins, is accelerated and it is then unnecessary to include them in the diet. Unfortunately, this investigation was interrupted before it was completed, and there has been no opportunity to reexamine the dietary role of

biotin, inositol, p-aminobenzoic acid, or of any of the more recently discovered vitamins. It is hoped that the study can be resumed at a later date.

SUMMARY

1. Hamsters grow at a normal rate and attain normal mature weights on simplified rations. The list of vitamins in the simplest satisfactory diet includes A, D, E, K, thiamine, riboflavin, pyridoxine, and pantothenic acid.

2. If vitamin E is omitted from the diet the animals collapse and die in 4 to 18 weeks. They may be rescued shortly after collapse by administration of vitamin E.

3. If vitamin K is omitted from the diet the rate of growth is irregular but the animals reach maturity in nearly normal time. During the period of arrested growth the animals develop small hemorrhagic areas.

4. If vitamins E and K are both omitted from the diet the animals apparently die from the vitamin E deficiency but they are also severely hemorrhagic.

5. A high percentage of females bore at least one litter on simplified diets that contained nicotinic acid, choline, and inositol, in addition to the vitamins required during growth. The data are insufficient to decide whether biotin or p-aminobenzoic acid are essential for the hamster.

6. Few females bore a second litter and it was concluded that the hamster requires at least one unrecognized vitamin for reproduction.

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THE EFFECT OF GERMINATION, THE STAGE OF MATURITY, AND THE VARIETY UPON THE NUTRITIVE VALUE OF SOYBEAN PROTEIN[1]

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(Received for publication September 25, 1943)

The biochemical literature contains numerous reports on the effect of heat on the nutritive value of proteins. Most of the investigators found heat to be injurious; however, in the case of legume protein, heat was found to be beneficial. The reader is referred to papers by Morgan and Kern ('34), Greaves and Morgan ('34), and Fairbanks and Mitchell ('35) for references to the effect of heat on animal protein. Results with legume protein are given in detail in papers by Hayward, Steenbock and Bohstedt ('36), and Johnson, Parsons and Steenbock ('39).

Much of the work on the effect of heat has been limited to soybeans of the Illini variety. This made it desirable to determine if this effect would be produced in other varieties as well. Since the improvement in nutritive value seemingly involved the sulfur-containing amino acids, five varieties of soybeans reported to vary widely in cystine content (Hamilton and Nakamura ('40)) were selected for the experiments. These included the Illini, Dunfield, Mansoy, Virginia Brown and Mandarin varieties.

EXPERIMENTAL

All the beans were fed in a ration of the following composition: soybeans to 10% protein (N $\times$ 5.7); salts (Wesson, '32), 4%; cottonseed oil,[3] 5%; glucose[4] to 100 parts; haliver oil, 2 drops weekly; riboflavin, 20 μg. daily; and a commercially available rice-bran concentrate,[5]

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. This work was supported in part by funds furnished by the Wisconsin Alumni Research Foundation.

[2] Now at Wayne University, Detroit, Michigan.

[3] Wesson oil.

[4] Cerelose.

[5] Vitab, from the Oil Products Company, Harrison, New Jersey.

400 mg. daily. The beans were fed raw and after autoclaving for 15 minutes at 17 pounds pressure to young male rats weighing between 55 and 65 gm. The efficiency of the protein was judged by the gain in weight per gram of protein ingested during an experimental period of 8 weeks. In addition, after the animals had been on the diets for 14 days, fecal samples were collected daily over a 6-day period to determine the percentage of ingested nitrogen which was absorbed.

Tao and Kamatsu ('32) have reported that the protein of the soybean is appreciably altered by germination, the globulins undergoing a partial hydrolysis with the formation of more simple forms such as proteoses and peptones. As there was a possibility that this might affect the nutritive value of the proteins, soybeans of the Illini variety were germinated and fed before and after autoclaving. For germination they were spread in a thin layer between moist layers of burlap contained in shallow porcelain pans. Sufficient distilled water was then added six to eight times daily to keep them moist, without actually immersing them in water. Germination was continued at 26°C. for approximately 60 hours. At the end of this time approximately all of the beans had sprouts varying in length from 2 to 12 mm. The beans were light yellow in color and about four times their original size. They were ground with a meat grinder, spread out thinly in trays and dried before a fan at room temperature. They were then reground to a fine meal in a burr mill with due care to prevent heating.

Parsons ('43) has suggested that the degree of maturity of the soybean might be a factor in determining the nutritive value of its protein. However, the data obtained were limited and disregarded possible varietal differences. Experiments were therefore carried out, using only beans of the Illini variety. These beans were picked when immature and shelled without the use of heat. They were ground and then dried before a fan at room temperature. Two preparations were fed, one unheated and one autoclaved.

RESULTS

The five varieties of beans when fed in the raw state failed to support normal growth, and all were greatly improved by autoclaving (table 1). Chemical determinations of the total sulfur by peroxide fusion indicated that the sulfur content of these varieties did not differ so widely as might have been assumed from a report on their cystine content (Hamilton and Nakamura, '40). Attempts to determine cystine revealed that the Hamilton and Nakamura technique, which involved an acid hydrolysis of NaOH extracts in the presence of large amounts

of nonprotein material, gave unreliable results[6] (Bailey, '37, and Lugg, '38).

While the percentage of nitrogen absorbed varied somewhat between animals of a given group the average figures were much alike for each of the five varieties studied. The values for the autoclaved beans were consistently higher than those obtained for the raw beans (3.9 to 6.1%). Again, differences between varieties were negligible. With respect to the efficiency of the raw protein to promote growth the Illini variety was somewhat superior. This superiority, however, did not carry over into the autoclaved series.

TABLE 1

A comparison of the nutritive value of the protein of five varieties of soybeans.

VARIETIES OF SOYBEANS	SULFUR CONTENT	BODY WEIGHT (AVERAGE)		NITROGEN ABSORBED		EFFICIENCY OF PROTEIN
		Initial	Final	Range	Average	Gram gain per gram consumed
Raw	%	*gm.*	*gm.*	%	%	
Illini	0.36	56.0	72.8	75.1–86.0	78.6	0.484
Mansoy	0.41	61.3	57.0	70.8–85.3	78.0	0.015
Dunfield	0.36	61.0	64.3	74.1–78.0	76.1	0.119
Virginia Brown	0.38	59.5	53.5	73.8–79.6	77.3	0.201
Mandarin	0.43	58.0	62.3	74.2–78.8	76.1	0.163
Autoclaved						
Illini	...	61.8	164.8	79.7–85.8	83.5	1.700
Mansoy	...	61.2	155.0	80.6–85.1	82.8	1.657
Dunfield	...	58.8	176.0	81.1–83.9	82.2	2.028
Virginia Brown	...	56.5	153.0	76.1–83.7	81.2	1.633
Mandarin	...	60.5	129.3	76.3–86.7	80.3	1.582

Four animals in each group.

Germinated and immature raw beans were distinctly superior to the mature bean. All three, however, were inferior to the corresponding autoclaved preparations. Especially noteworthy is the fact that while the percentage of nitrogen absorbed from the raw preparations is distinctly less than that absorbed from the autoclaved samples the values do not differ greatly. The efficiency of the protein consumed in promoting growth, however, ranged from 0.48 for the mature seed to 1.4 for the germinating seed (table 2). This supports the surmise that the differences between raw and heated mature beans are not due entirely to the improved digestibility of the latter.

[6] Unpublished data.

TABLE 2

The effect of heat on the nutritive value of Illini soybean protein.

SOYBEANS	BODY WEIGHT (AVERAGE)		NITROGEN ABSORBED	EFFICIENCY OF PROTEIN
	Initial	Final		Grams gain/grams consumed
Raw	gm.	gm.	%	
Mature	56.0	72.8	78.6	0.484
Germinated	58.0	136.2	...	1.504
(3 preparations)	62.4	128.2	...	1.415
	60.2	117.5	75.7	1.299
Immature	60.5	127.2	79.5	1.101
Autoclaved				
Mature	61.8	164.8	83.5	1.700
Germinated	57.3	185.0	83.2	1.903
Immature	60.5	211.5	87.8	2.013

Six animals in each group.

SUMMARY

Five varieties of soybeans including the Illini, Dunfield, Mansoy, Virginia Brown, and Mandarin varieties were studied, heated and unheated, for the nutritive value of their protein. No appreciable varietal differences were observed. All varieties were greatly improved by heating.

The protein of freshly germinated Illini beans was superior in nutritive value to the protein of the unheated mature beans, although the percentage of nitrogen absorbed was not increased.

Immature soybeans likewise supplied protein of higher nutritive value than that of raw mature beans. The protein of germinated as well as of immature soybeans was improved by heating.

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A SHORT METHOD OF CALCULATING THE NUTRITIVE VALUE OF DIETS *(concluded)*

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(Received for publication August 10, 1943)

Food habits of representative sections of the civilian population in this country have been discussed in detail by Stiebeling and Phipard, ('39). In connection with the nutritional evaluation of food provided for the soldier, an opportunity of obtaining an indication of military food habits has presented itself. Since the ration provided for the soldier subsisting on Field Ration A is equivalent roughly to a liberal civilian diet, it is of particular interest to note which foods are the most popular, and the approximate quantities of each which have been planned per day for each man. Furthermore, such information is of particular current interest, since it is as indication of which foods are likely to be made available for the Army.

In addition, the food pattern derived from these quantities of food forms the basis for a short method of nutritional evaluation of food as purchased. The method is based on the classification (Howe, Pritchett and Berryman, '42) of all nutritionally important foods into fifteen food groups, with the subsequent development of caloric, protein, mineral and vitamin values for each group, on the basis of quantitative use of the component items of the group. It is the purpose of this communication to present a discussion of food habits, and their relation to nutritive values for food groups.

The data presented herein have been obtained from records of food planned for the average soldier during the year May 1941 to April 1942, inclusive. The nutritive values for the first 6 months May to October 1941 have been called, for convenience, "summer" values, while those for November 1941 to April 1942, also presented here, are designated as "winter" values. The background of the methods used in computing these values, and of developing group values for use in connection with the short method of nutritional evaluation, has already been discussed (Berryman and Chatfield, '43).

During the period under discussion, quantities of food as shown in table 1 were planned for the soldier (pounds per man per day). Nutritional evaluation of the food indicates that it could be expected to contain approximately the amounts of nutritive components given in table 2.

TABLE 1

Pounds per man per day of foods prescribed for the soldier.

	SPRING–SUMMER MAY 1941–OCT. 1941	FALL–WINTER NOV. 1941–APRIL 1942	AVERAGE
Meats, fish and poultry	.845	.856	.851
Eggs	.178	.151	.165
Milk and milk products (fluid equivs.)	1.085	1.028	1.057
Butter	.093	.091	.092
Fats, other	.085	.079	.082
Grain products	.734	.662	.698
(Bread)	(.449)	(.383)	(.357)
Legumes dry and nuts	.066	.054	.060
Sugars and syrups	.340	.301	.321
Vegetables, leafy green and yellow	.420	.441	.431
Tomatoes	.160	.133	.147
Citrus fruits	.210	.190	.200
Potatoes	.654	.697	.676
Vegetables, other	.301	.272	.287
Fruits, other than citrus	.410	.322	.366
Fruits, dried	.034	.033	.034

TABLE 2

Nutritive components per man per day provided by foods in table 1.

	SPRING–SUMMER MAY 1941–OCT. 1941	FALL–WINTER NOV. 1941–APRIL 1942	AVERAGE
Calories	4320	4080	4200
Protein (gm.)	131	125	128
Fat (gm.)	195	189	192
Carbohydrate (gm.)	513	470	492
Calcium (mg.)	1012	960	986
Phosphorus (mg.)	2030	1940	1985
Iron (mg.)	24.5	23.6	24.1
Vitamin A (I.U.)	12900	14000	13450
Thiamine (mg.)	2.98 (2.00)[1]	3.29 (2.25)[1]	3.14 (2.13)[1]
Riboflavin (mg.)	2.50 (2.10)[1]	2.37 (2.00)[1]	2.44 (2.05)[1]
Nicotinic acid (mg.)	26.6 (20.7)[1]	26.9 (2.10)[1]	26.8 (20.9)[1]
Ascorbic acid (mg.)	137 (102)[1]	146 (104)[1]	142 (103)[1]

[1] Represents values after conservative deductions are made for probable losses due to cooking, preparation, etc.

Consideration of the quantities of individual food items planned for use by the soldier shows that certain foods usually predominate within a group. It is these particular items which undoubtedly will continue to be needed for military use within this country, provided they are available at all. Furthermore, the items which predominate have the greatest influence on the nutritive value of the food group involved.

Detailed presentation of the food pattern for the soldier follows in table 3; included are the levels for the two periods under discussion, namely: Spring and Summer 1941, and Fall and Winter 1941–1942. In addition, the values for Fall and Winter of 1942–1943 are presented for comparison purposes. The amounts of the various food items are presented as percentages of the food group, with the quantity of each food group being listed in pounds at the end of the group. "Miscellaneous" comprises those individual foods which were used in small quantities, i.e., lower than the lowest percentage figure listed.

Inspection of the various quantities of food items used indicates that the quantitative food pattern has not changed markedly, with the exception of certain items such as fresh corn, watermelon, cantaloupes, honeydew melon, apples, etc., the use of which obviously varies with the season. Other notable exceptions are boneless beef, which appears at a zero level in the 1943 table, and certain vegetables, the use of which has been decreased as a result of experience with the food habits of the average soldier.

NUTRITIONAL EVALUATION

A method for dietary evaluation based on the food pattern for the 6 months, May to October 1941, has been described by Berryman and Chatfield, '43. For purposes of convenience, the group nutritive values obtained were designated as "summer" values, and it was planned to obtain similar group values for November 1941 to April 1942, to be designated as "winter" values. The basis for the latter would, of course, be the food pattern for those months as presented below;

A study of the "winter" food pattern, and comparison with that of the "summer" indicated, however, that the differences were not as great as had been anticipated. Except for minor variations, which may well be ignored in view of the limits of accuracy of any dietary evaluation based on average nutritive values, foods within the following food groups showed but little deviation in the quantities planned for the two 6-month periods: milk products; fats, other; grain products; legumes dry, and nuts; sugars and syrups. Furthermore, groups containing only one food obviously did not require recalculation. These groups are as

TABLE 3

Pattern of food supply for the United States Army.

FOOD	SPRING AND SUMMER MAY 1941-OCT. 1941	FALL AND WINTER NOV. 1941-APRIL 1942	FALL AND WINTER NOV. 1942-APRIL 1943
	%	%	%
Meats, Fish and Poultry			
Beef			
Carcass	18.86	19.35	38.37
Rounds	8.12	0.50	0.15
Boneless	0.08	13.59	
Total	25.06	33.44	38.52
Pork			
Ham, fresh	1.54	1.78	1.09
Boston Butts	1.75	2.20	2.51
Chops	0.24	0.04	0.12
Loin	2.66	5.15	4.36
Shoulder	0.84	1.40	0.66
Ham SC	7.48	5.60	7.48
Total	14.51	16.17	13.07
Veal	4.14	5.26	5.44
Bacon	7.67	6.19	6.01
Fowl			
Chicken	6.81	7.11	7.34
Turkey	2.26	2.26	1.80
Duck	0.40	0.65	0.38
Total	9.47	10.02	9.52
Fish	6.92	6.90	4.46
Sausages	6.82	6.72	5.97
Miscellaneous	25.41	13.30	17.01
Pounds/man day	0.85	0.86	0.93
Eggs, fresh	100.	100.	100.
Pounds/man day	0.18	0.15	0.16
Milk products (fluid equivalents)			
Fresh milk	46.21	48.42	46.49
Evaporated	34.56	36.10	35.59
Cheese	12.24	12.89	14.98
Ice cream	5.77	2.10	2.20
Miscellaneous	1.22	0.49	0.74
Pounds/man day	1.06	1.03	1.11
Vegetables, leafy green or yellow (fresh and canned)			
Cabbage	22.11	17.47	15.91
Carrots	11.04	12.80	14.11
Lettuce	16.07	11.91	10.93
Peas	9.57	9.19	7.67
Sweet potatoes	8.97	12.82	15.16
String beans (canned and fresh)	10.78	9.19	9.17
Spinach (canned)	7.54	3.88	1.53
Miscellaneous	13.92	22.74	25.52
Pounds/man day	0.42	0.44	0.45
Tomatoes, etc.			
Tomatoes, canned	49.66	56.68	48.73
Tomatoes, fresh	38.05	21.11	26.03
Tomato juice	3.24	10.11	13.37
Tomato catsup	7.86	9.36	10.32
Miscellaneous	1.19	2.74	1.55
Pounds/man day	0.16	0.13	0.14
Citrus Fruits			
Grapefruit, fresh	32.76	41.32	37.47
Lemons	22.51	8.00	10.52
Oranges, fresh	44.21	46.16	49.24
Miscellaneous	0.52	4.52	2.77
Pounds/man day	0.21	0.19	0.28

FOOD	SPRING AND SUMMER MAY 1941-OCT. 1941	FALL AND WINTER NOV. 1941-APRIL 1942	FALL AND WINTER NOV. 1942-APRIL 1943
	%	%	%
Potatoes, white	100.	100.	100.
Pounds/man day	0.65	0.70	0.67
Butter	100.	100.	100.
Pounds/man day	0.093	0.099	0.08
Other fats			
Lard or lard sub.	73.79	78.17	74.90
Mayonnaise	21.84	18.02	21.01
Miscellaneous	4.37	3.81	4.09
Pounds/man day	0.08	0.08	0.07
Grain Products			
Bread, issue	60.94	57.84	53.52
Flour, issue	19.58	21.83	27.04
Cereals, dry prepared	4.27	3.59	3.55
Miscellaneous	15.21	16.74	15.89
Pounds/man day	0.74	0.66	0.67
Legumes, dry (beans, peas, etc.)			
Navy beans	36.21	42.96	42.80
Black eyed peas	7.73	10.56	5.17
Lima beans	25.45	23.70	23.06
Kidney beans	16.06	11.11	15.31
Others	14.55	11.67	13.66
Pounds/man day	0.07	0.05	0.05
Sugars and Syrups			
Sugar, granulated	76.02	73.08	71.71
Syrup	10.44	10.47	10.03
Jam-preserves	9.95	8.84	6.63
Miscellaneous	3.59	7.61	11.63
Pounds/man day	0.34	0.30	0.26
Vegetables other than leafy green or yellow			
Beets, canned and fresh	10.48	12.93	10.94
Celery	13.07	15.06	15.59
Corn, canned	11.71	11.79	12.03
Corn, fresh	8.98	0.33	0.47
Onions	25.62	27.03	25.37
Turnip roots	4.62	8.25	6.80
Sauerkraut, canned	5.85	4.94	5.23
Miscellaneous	19.67	19.67	23.57
Pounds/man day	0.30	0.27	0.28
Fruits other than Citrus (fresh and canned)			
Apples, fresh	16.35	31.63	46.55
Bananas	10.81	19.19	6.93
Peaches, fresh	6.66		0.38
Cantaloupe, fresh Honeydew, fresh Watermelon, fresh	17.61	0.53	0.31
Apples, canned	11.62	9.43	5.50
Peaches, canned	8.59	7.53	6.86
Pineapple, canned	6.32	6.94	4.13
Miscellaneous	22.04	24.75	29.34
Pounds/man day	0.41	0.32	0.29
Fruits, Dried			
Apples	2.05	2.75	8.61
Apricots	14.04	16.21	8.01
Peaches	16.37	14.68	8.31
Prunes	33.92	23.55	27.00
Raisins	33.62	41.90	47.48
Miscellaneous	0.0	0.91	0.59
Pounds/man day	0.03	0.03	0.03

follows: eggs; fats, butter; potatoes, white. The number of groups remaining closely similar in the distribution of their component items or requiring no change from previous calculation was therefore eight; this left seven food groups which were nutritionally significant and which required further calculation based on the food pattern of the second 6 months.

The tables of weighted "winter" and "summer" nutritive values for the various classes of food are presented in tables 4 and 5 respectively.

It then became of interest to speculate on the possible development of an annual table which might be usable without regard to season. Numerous calculations were made between the accuracy obtained by the long method of calculation (i.e., using individual food values) and the contemplated "annual" table. Furthermore, the accuracy of the latter was compared with that of the winter and summer tables. While it appeared that such a table would give an evaluation which would be reasonably valid in view of the wide possible variation of nutrients in various species of foods, yet a satisfactory degree of accuracy in calculation was not obtainable. This was particularly true in the case of ascorbic acid and Vitamin A, due primarily to seasonal changes in the availability and use of certain fruits and vegetables. It is therefore considered advisable to use separate tables for the winter and summer months (table 4 and table 5), in preference to a single table for year-around use.

DISCUSSION

As stated above, the values for Vitamins A and C in the "summer" and "winter" tables show appreciable differences in some cases. The major reasons for the variations in the two sets of values have been investigated, and are presented below in some detail.

Vitamin A

In so far as Vitamin A is concerned, the chief differences lie in the changing distribution of items in the following groups: leafy green and yellow vegetables, tomato products, and fruits other than citrus. The values for Vitamin A per pound of these food groups are as follows (taken from tables 4 and 5).

Vegetables, leafy green and yellow

May–October 1941	November 1941–April 1942
14750 I.U. per lb.	16,100 I.U. per lb.

The one item contributing chiefly to this change is sweet potatoes, which in terms of percentage, showed an increase of 8.97 to 13.52%.

TABLE 4

Short method of computing nutritive value of ration — (Winter).

Nutrients per pound of food groups "as purchased" (based on food prescribed for U. S. Army Nov. 1941–April 1942).

GROUP	CALORIES	PROTEIN	FAT	C(OH)	Ca	P	Fe	VITAMIN A	THIAMINE	RIBOFLAVIN	NICOTINIC ACID	ASCORBIC ACID
		gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*
Meats, fish and poultry	1115	66	95	2	42	728	10.4	117 [1]	1.93 [2]	.79 [1]	19.9	4
Eggs, fresh	635	52	46	3	218	848	10.9	4040	.56	1.45	.2	0
Milk products (equivs.)	360	16	21	25	556	427	.9	1020	.11	.74	.4	4
Fats, butter	3320	3	367	2	73	73	.9	14970	.01	.04	.5	0
Fats, other	3900	1	432	3	2	9	.1	120	.01	.01	.2	0
Grain products [4]	1360	40	14	270	160	482	9.0 [3]	20	1.12 [3]	.83 [3]	11.2 [3]	0
Legumes, dry and nuts	1630	97	15	276	538	1968	40.7	10	2.40	1.33	12.5	0
Sugars and syrups	1680	1	0	420	37	16	2.2	0	.00	.00	0.0	0
Vegetables, leafy green and yellow	173	6	1	35	157	158	3.4	16100	.31	.26	2.7	116
Totatoes	138	5	1	29	64	179	2.5	3335	.36	.20	2.0	69
Citrus fruits	147	2	47	33	84	66	1.1	96	.26	.08	1.6	121
Potatoes, white	330	8	0	73	30	186	2.8	150	.34	.19	4.5	46
Vegetables, other	190	6	1	39	116	175	2.1	155	.13	.23	1.0	64
Fruits, other than citrus, fresh and canned	330	2	1	78	34	54	1.4	1050	.09	.19	1.5	18
Fruits, dried	1300	13	2	307	253	453	17.7	8130	.47	.40	6.8	0

[1] When liver is used add 125,000 I.U. Vitamin A and 14.56 mg. of riboflavin per pound of liver.

[2] Use this figure only when the combined weight of the lean pork cuts (viz., fresh and cured ham, pork loin and chops, Boston butt, and shoulder) is 15% or more of the weight of the meat group. When the combined weight is less than 15%, compute the thiamine thus: (wt., in lbs., of lean pork cuts × 5.9) plus (wt., in lbs., of all other meat × 1.0). This gives the quantity of thiamine in milligrams in the entire group.

[3] When enriched flour and bread are not used, count thiamine in the entire group as only 0.59 mg. per pound, riboflavin 0.29 mg. per pound, nicotinic acid 4.2 mg. per pound, and iron 5.5 mg. per pound (based on straight flour); no changes need be made in other nutrients.

[4] Values based on new enrichment levels effective October 1, 1943.

TABLE 5

Short method of computing nutritive value of ration (Summer).
Nutrients per pound of food groups "as purchased" (based on food prescribed for U. S. Army May–October 1941).

GROUP	CALORIES	PROTEIN	FAT	C(OH)	Ca	P	Fe	VITAMIN A	THIAMINE	RIBOFLAVIN	NICOTINIC ACID	ASCORBIC ACID
		gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*
Meats, fish and poultry	1120	65	95	2	41	719	9.8	120 [2]	1.87 [2]	.77 [1]	19.4	3
Eggs, fresh	635	52	46	3	218	848	10.9	4040	.56	1.45	.2	0
Milk products (equivs.)	360	16	21	25	556	427	.9	1020	.11	.74	.4	4
Fats, butter	3320	3	367	2	73	73	.9	14970	.01	.04	.5	0
Fats, other	3900	1	432	3	2	9	.1	120	.01	.01	.2	0
Grain products [4]	1360	40	14	270	160	482	9.0 [3]	20	1.12 [3]	.83 [3]	11.2	0
Legumes, dry and nuts	1630	97	15	276	538	1968	40.7	10	2.40	1.33	12.5	0
Sugars and syrups	1680	1	0	420	37	16	2.2	0	.00	.00	.0	0
Vegetables, leafy green and yellow	150	7	1	30	145	152	3.5	14750	.29	.24	.27	91
Tomatoes	130	5	1	26	61	160	2.6	3860	.36	.20	2.2	78
Citrus fruits	140	2	1	32	91	66	1.2	90	.23	.07	1.5	118
Potatoes, white	330	8	0	73	30	186	2.8	150	.34	.19	4.5	46
Vegetables, other	180	6	1	37	102	173	1.9	180	.12	.22	.9	49
Fruits, other than citrus, fresh and canned	290	2	1	69	34	51	1.3	1940	.09	.17	1.4	24
Fruits, dried	1280	12	2	302	245	452	18.0	8320	.47	.60	6.6	0

[1] When liver is used add 125,000 I.U. Vitamin A and 14.56 mg. of riboflavin per pound of liver.

[2] Use this figure only when the combined weight of the lean pork cuts (viz., fresh and cured ham, pork loin and chops, Boston butt, and shoulder) is 15% or more of the weight of the meat group. When the combined weight is less than 15% compute the thiamine thus: (wt., in lbs., of lean pork cuts × 5.9) plus (wt., in lbs., of all other meat × 1.0). This gives the quantity of thiamine in milligrams in the entire group.

[3] When enriched flour and bread are not used, count thiamine in the entire group as only 0.59 mg. per pound, riboflavin 0.29 mg. per pound, nicotinic acid 4.2 mg. per pound, and iron 5.5 mg. per pound (based on "straight" flour); no changes need be made in other nutrients.

[4] Values based on new enrichment levels effective October 1, 1943.

In terms of International Units per pound, this is equivalent to an increase of approximately 820, or more than half of the change in the value per pound for the group. Other cumulative increases in the quantities of such items as carrots, squash, pumpkin, turnip greens, etc., with the concomitant decrease in the use of items relatively dilute with regard to Vitamin A (i.e., cabbage, lettuce, etc.), resulted in further increase in the overall Vitamin A value of the group for the winter months.

Tomatoes

May–October 1941	November 1941–April 1942
3860 I.U. per lb.	3335 I.U. per lb.

The drop in the use of fresh tomatoes was primarily responsible for these changes. A drop of approximately 17% in the use of this item, is equivalent to a change in terms of I.U. per pound of approximately 1400. Part of this change was offset by the increased percentage use of canned tomato products; it was not entirely offset, however, because of the lower Vitamin A value of the latter. The net result was, therefore, a drop of about 500 I.U. per pound in the winter value.

Fruits other than citrus

May–October 1941	November 1941–April 1942
1940 I.U. per lb.	1050 I.U. per lb.

The items responsible for this change are the various types of melons (cantaloupe, honeydew) and fresh peaches, the use of which during the winter months decreased concurrently with an increase in the use of Vitamin A dilute items such as apples, bananas, etc.

Vitamin C

The changes in Vitamin C values per pound were greatest in the "leafy green or yellow vegetable" and "other vegetables" groups.

Vegetables, leafy green and yellow

May–October 1941	November 1941–April 1942
91 mg. per lb.	116 mg. per lb.

The items responsible for the change in Vitamin C values per pound of leafy green-yellow vegetables are not, as might be first considered, the change in sweet potatoes, since the increased use of this item was approximately offset with regard to Vitamin C by a decrease in the use of cabbage. Rather the small but cumulative increases in Vitamin C rich items such as turnip greens, spinach, broccoli, green peppers, etc., became of importance when considered together with the simultaneous

decrease in the use of lettuce and other items which are less rich in the vitamin.

Vegetables other than leafy green and yellow.

May–October 1941	November 1941–April 1942
49 mg. per lb.	64 mg. per lb.

The increased use of the following, together with a decreased use of fresh corn and other Vitamin C dilute items were responsible for the change in the value per pound for the group: green lima beans; cauliflower; green onions; rutabaga; and turnips.

Boneless versus carcass beef

The question is frequently raised as to what correction should be made when large quantities of boneless beef are used. Since the use of boneless beef during 1943 has been negligible within this country, the matter probably does not warrant detailed discussion. However, the answer to be given to such a question is that usually no correction is needed. One example will serve to illustrate the point. If all of the quantity of boneless beef used during November 1941 to April 1942 were converted to a carcass beef equivalent by use of the ratio 1:1.4, the result would be a change from .1163 pounds per man per day of boneless beef to .1628 pounds per man per day of carcass beef equivalent. The change in percentage contribution of carcass beef would be from 19.35 to 38.3, with the boneless percentage, of course, being zero. (This situation is analogous to that observed for the same items during November 1942 to April 1943). The error (per pound of "meats, fish, and poultry") involved by using the values as they now stand would be as follows:

CALS.	PROT.	FAT	C(OH)	Ca	P	Fe	A	THIAMINE	RIBOFLAVIN	NICOTINIC ACID	ASCORBIC ACID
	gm.	*gm.*	*gm.*	*mg.*	*mg.*	*mg.*	*I.U.*	*mg.*	*mg.*	*mg.*	*mg.*
32	2.2	2.2	0	1.2	23.4	.33	0	.02	.02	0.6	0

Such error in calculation is obviously of the slightest magnitude, and not within the province of the intended purpose of a method of evaluating the approximate nutritive value of the diet.

SUMMARY

The food pattern planned for the soldier subsisting on Field Ration A during the year May 1941 to April 1942 has been described. Seasonal tables of nutritive values based on this food pattern have been presented as a rapid means of calculating the probable nutritive value of food

supplied for Field Ration A, the rough equivalent of a liberal civilian diet. Reference is made to a previous publication for the detailed background of such tables, and for the degrees of accuracy made possible by their use.

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HUMAN RESPIRATORY QUOTIENTS IN RELATION TO ALVEOLAR CARBON DIOXIDE AND BLOOD LACTIC ACID AFTER INGESTION OF GLUCOSE, FRUCTOSE, OR GALACTOSE [1]

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(Received for publication September 24, 1943)

The changes in the respiratory quotient after the ingestion of hexoses have been studied by many investigators, and attempts have been made to ascertain whether the rises in respiratory quotient are ascribable to a combustion of the sugar, to a formation of fat from the sugar, to a change in the acid-base condition of the blood with a resultant displacement of carbon dioxide, or to a combination of these three processes. Nevertheless it still remains an unsettled question whether there are any real differences in the effects on the respiratory quotient of the ingestion of the three principal hexoses, glucose, fructose, and galactose. The object of the present research was to determine the respiratory quotients of human subjects after ingestion of standard amounts of these three hexoses taken separately, and periodically to ascertain the lactic acid content of the blood. Simultaneously with the measurement of the respiratory exchange, samples of alveolar air were drawn for analysis of the carbon dioxide content.

While our research was in progress, Bachmann and Haldi ('37) reported the results of a study in which two of these sugars were given to three male adults and determinations made of the carbon dioxide and the lactic acid in blood. After the ingestion of 50 gm. of glucose, there was no increase in the lactic acid and no decrease in the carbon-dioxide content of the blood, and they concluded that the respiratory quotients obtained within 30 minutes after the ingestion of glucose were true metabolic quotients. The same quantity of fructose led to an in-

[1] A preliminary report of this study was presented before the American Institute of Nutrition, Memphis, Tennessee, April 21, 1937 (Carpenter, Bensley, Dill, and Edwards, '37).

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crease in the lactic acid and a decrease in the carbon dioxide content of the blood. They believe that the respiratory quotients after ingestion of fructose are not true metabolic quotients. When correction was made for the changes in lactic acid and carbon dioxide in the blood, the quotients obtained within 30 to 45 minutes after ingestion of fructose were considerably higher than those obtained in a corresponding period after ingestion of glucose, and they were led to the conclusion that relatively more carbohydrate was burned after the ingestion of fructose or that this sugar was transformed into fat.

Stöhr ('33) found that 0.2 gm. of glucose per 100 gm. of body weight, given to rats that had been 24 hours without food, resulted in a lowering of the alkali reserve after 15 to 30 minutes, but that this reserve returned to normal after 1 hour. Fructose had no effect in this respect. Sterkin and Wengerowa ('34) observed a marked rise in the lactic acid of the blood of dogs after oral ingestion of 2 gm. of fructose per kilogram of body weight, but after glucose only variations in either direction. Lactose produced no change. A study of Blatherwick, Bradshaw, Ewing, and Sawyer ('40) showed that, 1 hour after ingestion by stomach tube, fructose produced with rats more than a 50% increase in the lactic acid content of liver and muscles as compared with the results when glucose was given. These findings they consider as evidence that lactic acid was largely responsible for the higher respiratory quotients after fructose. Sachs, Sternfeld, and Kraus ('42) found no rise in the lactic acid of the blood after oral ingestion of 50 gm. of fructose in two boys who showed essential fructosuria, whereas with two normal controls they found marked rises within 30 and 65 minutes after ingestion.

EXPERIMENTAL

The subjects of the experiments were two men, H. C. W. (age, 21 years; height, 176 cm.; weight, 69.4 kg.) and W. C. (age, 19 years; height, 170 cm.; weight, 70.3 kg.). The subject came to the laboratory in the post-absorptive condition and sat in a comfortable chair for half an hour. He then began breathing in the respiration apparatus, and during the next hour the respiratory exchange was measured in three consecutive 15-minute periods. Alveolar air samples were taken simultaneously at approximately 5-minute intervals. After these three basal periods, the subject released the mouthpiece of the respiration apparatus, and a sample of venous blood was drawn with the subject sitting. The dose of sugar for the day was then given, following the ingestion of which the subject continued breathing in the respiration apparatus for

two or three more consecutive 15-minute periods, and alveolar air samples were again taken. Another blood sample was drawn, two or three more 15-minute periods were run, and so on until a total of approximately 4 hours had elapsed after the dose was given. The amount of sugar given was 50, 75, or in most instances 100 gm. This was taken in 310 ml. of water, after which the subject drank the small amount of water (25 ml.) used to wash the beaker and tube. In no-dose control experiments the same routine of period measurements was carried out.

The respiratory exchange was determined by the use of the mouthpiece and open-circuit apparatus of Carpenter and Fox ('31), and the alveolar air samples were obtained by the technique of Carpenter and Lee ('33a). All samples of expired air and of alveolar air were analyzed in duplicate by means of the portable Haldane gas-analysis apparatus. The blood samples were analyzed for lactic acid by the method of Edwards ('38).

RESULTS

Alveolar carbon dioxide

The results of the alveolar carbon dioxide determinations are given in table 1, expressed as the average partial pressures in millimeters noted in the preliminary 45 minutes of observation before sugar ingestion and in the several groups of periods after the dose. In the control experiments, the alveolar carbon dioxide of H. C. W. was slightly lower in general throughout the series than in the preliminary 45 minutes. With W. C. there was but little difference in the alveolar carbon dioxide, although there was a slight tendency for it to fall in the course of the 4 hours. In the experiments with glucose, there was an average drop with H. C. W. from 42.0 to 41.0 mm. at the end of the experiments, but with W. C. there was practically no change in the entire series. In the fructose experiments, there was little change with H. C. W. and with W. C. a drop of about 1 mm. In the galactose experiments with H. C. W. there was at first a slight rise, then a return to the pre-ingestion level (average, 42.8 mm.), and thereafter throughout the rest of the experiment a decided drop, the final average value for the two experiments being 40.2 mm. Similarly with W. C., there was a drop from the average initial level of 39.4 mm. to 37.9 mm. at the end of the experiments. With H. C. W. the lowest alveolar carbon dioxide came at the end of the galactose experiments, whereas with W. C. it occurred in the period from 135 to 180 minutes after the dose. These findings accord with the results obtained by one of us (Carpenter, '37; Carpenter and Lee, '33b) in pre-

vious studies, in which no significant change was noted in the alveolar carbon dioxide of a trained human subject after ingestion of 25 gm. of fructose or glucose, whereas a marked decrease was observed after ingestion of 25 gm. of galactose. In the earlier experiments the peak of the change in alveolar carbon dioxide was reached much earlier than in the present series of experiments, in which the dose was 100 gm.

TABLE 1

Average alveolar carbon dioxide before and after ingestion of glucose, fructose, or galactose (millimeters partial pressure).

SUBJECT AND TYPE OF EXPT.	DATE (1936)	PRELIM. 45 MIN.	MINUTES AFTER DOSE [1]				
			0–45	45–90	90–135	135–180	180–240
H. C. W.							
Control	April 28	42.6	41.1	40.9	40.8	39.8	40.5
	May 7	43.7	42.3	41.8	42.0	41.8	41.9
	May 21	42.7	43.0	42.6	42.4	42.8	43.0
	June 5	41.2	40.9	41.0	40.7	40.6	40.6
Glucose	May 19	42.4	42.0	41.5	41.0	41.2	40.7
	June 1	41.6	42.3	42.4	41.7	41.3	41.3
Fructose	May 14	42.5	42.5	41.8	41.3	41.5	42.0
	May 28 [2]	41.9	42.2	41.1	41.2	41.6	41.7
Galactose	May 12	42.1	43.1	41.3	41.2	40.6	39.1
	May 23	43.5	44.0	43.6	42.3	41.5	41.2
W. C.							
Control	May 26 [3]	(38.9)	(37.2)	(37.4)	(34.1)	(35.8)	(36.0)
	June 3	36.8	36.5	36.0	36.1	35.7	35.2
	June 18	37.7	38.2	38.5	38.1	37.4	38.0
	July 3	40.2	40.0	40.2	39.8	40.0	39.5
Glucose	June 8	36.2	36.3	35.9	36.3	35.6	35.2
	June 19	37.4	37.6	37.9	38.4	37.6	38.4
Fructose	June 12 [2]	37.1	36.5	36.2	37.1	37.5	37.2
	June 24 [4]	39.5	38.4	37.4	37.8	38.7	38.7
	June 26	40.7	40.3	38.6	38.9	38.7	39.5
	June 29	41.8	41.6	40.1	39.9	39.9	39.6
Galactose	June 10	37.3	37.0	36.2	36.0	35.8	36.1
	June 22	39.7	39.2	38.4	38.7	37.6	38.4
	July 1	41.1	41.6	39.7	39.1	38.4	39.1

[1] 100 gm. of sugar, unless otherwise noted.

[2] 50 gm. of sugar.

[3] This was W. C.'s first experience in having alveolar air samples taken.

[4] 75 gm. of sugar.

Blood lactic acid

The data regarding lactic acid in the blood are given in table 2. In the control series the most striking changes occurred in the first experiment with H. C. W., on April 28. The procedure for taking the blood samples in this experiment was different from that in any of the following experiments because, instead of remaining seated, the subject rose from the chair and reclined on a couch before the sample was taken.

TABLE 2

Lactic acid in blood before and after ingestion of glucose, fructose, or galactose (milligrams per 100 ml.).

SUBJECT AND TYPE OF EXPT.	DATE (1936)	BASAL	MINUTES AFTER DOSE [1]				
			45 ±	90 ±	135 ±	180 ±	240 ±
H. C. W.							
Control	April 28	(9.7)	(10.5)	(9.7)	...	(11.2)	...
	May 7	7.4	6.7	6.7	6.7	6.7	...
	May 21	8.2	7.5	6.0	6.7	6.0	...
	June 5	6.7	6.7	7.5	8.2	7.5	7.5
Glucose	May 19	8.2	11.2	9.0	9.7	9.7	...
	June 1	7.5	11.2	9.0	9.0	12.7	8.2
Fructose	May 14	9.0	21.7	19.5	15.7	10.5	...
	May 28 [2]	6.7	15.7	10.5	9.0	7.5	6.7
Galactose	May 12	6.7	12.0	15.0	12.0	12.0	...
	May 23	6.7	11.2	10.5	10.5	11.2	9.0
W. C.							
Control	May 26	11.2	8.2	6.7	6.7	7.5	...
	June 3	6.0	6.0	9.0	9.0	7.5	6.7
	June 18	7.5	8.2	6.7	7.5	6.7	7.5
	July 3	8.2	7.5	7.5	6.7	6.0	6.7
Glucose	June 8	6.7	10.5	9.7	8.2	8.2	8.2
	June 19	8.2	11.2	11.2	10.5	10.5	9.0
Fructose	June 12 [2]	7.5	15.7	16.5	13.5	9.0	8.2
	June 24 [3]	8.2	15.0	15.0	12.7	9.7	8.2
	June 26	8.2	19.5	19.5	18.7	12.0	7.5
	June 29	8.2	15.7	19.5	15.7	12.0	7.5
Galactose	June 10	7.5	15.0	15.0	12.7	10.5	7.5
	June 22	7.5	21.0	16.5	17.2	9.0	6.7
	July 1	8.3	15.0	15.0	14.2	9.7	6.0

[1] 100 gm. of sugar, unless otherwise noted.

[2] 50 gm.

[3] 75 gm.

This muscular movement was great enough so that it not only raised the general level of the lactic acid in the blood of this subject in this particular experiment compared with the levels noted with the same subject in the other control experiments, but it also produced irregularity in the results, as the range in the values is from 9.7 to 11.2 mg. per 100 ml. In the other control experiments with this subject there is but little irregularity in the values on any individual day, and in general the values on the different days agree fairly well with each other. In the case of W. C. the first blood sample he had ever had taken was that on May 26th. Therefore the higher value in the first sample on this day may be ascribable to greater activity and the slight excitement coincident with the procedure of taking the sample. The subsequent values on that day are not irregular, and for the most part the range in the values on the other 3 days is not wide, with the exception of the two values of 9.0 mg. on June 3rd. The cause for these being higher than the rest of the values is not known.

In the glucose experiments there is a decided rise in the lactic acid values within 45 minutes after the dose. This does not agree with the results of Bachmann and Haldi ('37), who found no change in the blood lactic acid after 50 gm. of glucose. However, as the rise with our subjects was so large and this large rise was found in all four experiments, there is no doubt about its validity. With H. C. W. the values after the forty-fifth minute show little real indication of a rise, with the exception on June 1st of the value of 12.7 mg. in the periods from 135 to 180 minutes after the sugar. With W. C. on June 19th, however, the values remain higher than the pre-ingestion level throughout the experiments. In the fructose series immediately after the dose was given, whether the amount was 50, 75, or 100 gm., there was a marked rise in the lactic acid content of the blood. With H. C. W. on May 14th, the blood lactic acid after 100 gm. of fructose remained higher than the pre-ingestion level throughout the experiment. On May 28th, however, the rise with the same subject after 50 gm. was not so long and was entirely over at the end of 3 hours. With W. C. doses of 50 and 75 gm. caused a rise in the blood lactic acid for 135 minutes, whereas doses of 100 gm. caused a rise for 3 hours. In all four experiments with W. C. the lactic acid at the end of 4 hours had reached the pre-ingestion level. In all the galactose experiments there is a rise in the values immediately after ingestion of the sugar. In both experiments with H. C. W. the lactic acid remained above the pre-ingestion level for at least 3 hours. With W. C. the rise was even greater than that noted with H. C. W., and the lactic acid remained at a higher level until the end of 135 minutes. At 180 minutes

it had dropped practically to the pre-ingestion level, and at 240 minutes it had returned to the pre-ingestion level in all three experiments.

Corrections in respiratory quotients necessitated by changes in alveolar carbon dioxide and blood lactic acid

As these sugars all had definite effects, particularly upon the blood lactic acid and in the case of galactose upon the alveolar carbon dioxide, it became necessary to calculate the effect of the changes in lactic acid and alveolar carbon dioxide upon the respiratory exchange, especially the respiratory quotient. The calculation was made by correcting the carbon dioxide elimination for the change in alveolar carbon dioxide and for the theoretical change produced upon the carbon dioxide content of the blood by the rise or fall of the lactic acid content of the blood. In this calculation it was assumed: (1) that the production and removal of lactic acid are associated with equivalent changes in the carbon dioxide dissociation curve, that is, that a change of 1 millimole in the carbon dioxide combining power of blood is caused by a change in lactic acid concentration of 1 millimole or 9 mg. per 100 ml. — in other words, an increase of 1 mg. % in lactic acid concentration will reduce the carbon dioxide curve 0.247 volume %; (2) that the change in alveolar carbon dioxide tension of itself reflects accurately the change in arterial carbon dioxide tension (Bock and Field, '24); (3) that the change in the carbon dioxide content of the blood water equals the change in the carbon dioxide content of the body water (Irving, Foster, and Ferguson, '32); and (4) that the water of the blood equals 840 ml. per liter and the water of the body is equivalent to 50 liters. Hence a change of 1 volume % in the blood is equal to 1.19 ml. of carbon dioxide per 100 ml. of blood water, or a total of 595 ml. in the body.

The magnitude of the corrections necessary in the respiratory quotients according to calculations based on these assumptions is shown in table 3. In the control experiments lasting a total of 5 hours, there is little or no change in the respiratory quotient that may be ascribed to a change in the lactic acid content of the blood or to a change in the alveolar carbon dioxide, provided the experiments were carried out with extreme care so far as the activity of the subject was concerned. In the glucose experiments the largest correction would be expected in the first group of periods after the dose. With H. C. W. this is so on May 19th, the correction being — 0.04. On June 1st, however, the maximum correction occurs in the period from 135 to 180 minutes. With W. C. there is little or no really significant correction in the quotient for the

entire series, the maximum being ± 0.03. In the fructose experiments the corrections are somewhat larger. With H. C. W. the maximum corrections in the two experiments are — 0.10 and — 0.14 in the first group of periods from 0 to 45 minutes. There is also a significant correction in the reverse direction on May 14th, in the period from 135 to 180 minutes. With W. C. the corrections in the first group of periods vary from — 0.07 to — 0.15, and corrections of the order of ± 0.03 to ± 0.06 are found in the following periods, the signs changing from minus to plus in the groups of periods from 90 to 135 minutes and thereafter. In the

TABLE 3

Corrections in respiratory quotients based on changes in alveolar carbon dioxide and lactic acid.

SUBJECT AND TYPE OF EXPT.	DATE (1936)	MINUTES AFTER DOSE [1]				
		0–45	45–90	90–135	135–180	180–240
H. C. W.						
Control	April 28	— 0.04	+ 0.01	± 0.00	— 0.04	+ 0.02
	May 7	— 0.02	— 0.01	+ 0.01	— 0.01	± 0.00
	May 21	+ 0.02	+ 0.01	— 0.02	+ 0.02	— 0.01
	June 5	± 0.00	± 0.00	— 0.01	+ 0.02	± 0.00
Glucose	May 19	— 0.04	+ 0.01	— 0.01	+ 0.01	± 0.00
	June 1	— 0.02	+ 0.03	— 0.02	— 0.05	+ 0.03
Fructose	May 14	— 0.14	+ 0.01	+ 0.04	+ 0.06	+ 0.03
	May 28 [2]	— 0.10	+ 0.04	+ 0.01	+ 0.03	± 0.00
Galactose	May 12	— 0.04	— 0.07	+ 0.03	— 0.01	+ 0.02
	May 23	— 0.04	± 0.00	— 0.03	— 0.03	+ 0.02
W. C.						
Control	May 26	± 0.00	+ 0.02	— 0.06	+ 0.03	± 0.00
	June 3	— 0.01	— 0.04	± 0.00	± 0.00	± 0.00
	June 18	± 0.00	— 0.02	— 0.02	— 0.01	± 0.00
	July 3	± 0.01	+ 0.01	± 0.00	+ 0.01	— 0.01
Glucose	June 8	— 0.03	± 0.00	+ 0.03	— 0.01	± 0.00
	June 19	— 0.02	± 0.00	+ 0.02	— 0.02	+ 0.03
Fructose	June 12 [3]	— 0.11	— 0.01	+ 0.05	+ 0.06	+ 0.01
	June 24 [3]	— 0.11	— 0.02	+ 0.04	+ 0.05	+ 0.01
	June 26	— 0.15	— 0.03	+ 0.01	+ 0.06	+ 0.06
	June 29	— 0.07	— 0.06	+ 0.04	+ 0.04	+ 0.03
Galactose	June 10	— 0.09	— 0.02	+ 0.02	+ 0.02	+ 0.02
	June 22	— 0.16	+ 0.02	— 0.01	+ 0.07	+ 0.04
	July 1	— 0.08	— 0.04	± 0.00	+ 0.04	+ 0.04

[1] 100 gm. of sugar unless otherwise noted.

[2] 50 gm.

[3] 75 gm.

first two groups of periods the corrections are necessitated by increases in the lactic acid content of the blood, whereas in the remaining three groups they are ascribable to decreases in the lactic acid content, in which equilibrium in blood lactic acid is regained. In the galactose series with H. C. W. there is a correction of — 0.07 on May 12th, but this is the largest correction in any of the groups of periods with this subject. With W. C. the corrections in the group of periods after galactose, 0 to 45 minutes, are larger than in the succeeding periods. Thereafter little, if any, correction is necessary except on June 22nd, where there is a correction of + 0.07 in the group of periods from 135 to 180 minutes. The greatest corrections in the respiratory quotient ascribable either to lactic acid formation or to changes in alveolar carbon dioxide are those in the fructose experiments.

Net changes in respiratory quotients ascribable to sugar ingestion

When the respiratory quotients are thus corrected for the changes in the carbon dioxide and lactic acid contents of the blood, the resultant net changes in the quotients ascribable to the ingestion of the sugars (when the quotients after sugar ingestion are compared with the basal or pre-ingestion levels) are as shown in table 4. In the glucose series in the first group of periods after ingestion of glucose there is, on the average, little or no change ascribable to the sugar. In the second group of periods there is an average change of + 0.08 and in the third group, of + 0.11. In the last two groups of periods the average changes are the same with both subjects. The maximum increase in respiratory quotient after glucose ingestion is apparently reached in the periods between 90 and 135 minutes, but in no case is there a return to the pre-ingestion level in 4 hours. With fructose the course of the increases in the respiratory quotient is somewhat similar to that with glucose, except that beginning with the second group of periods, 45 to 90 minutes, the increases are larger than those found with glucose. In other words, after all the corrections necessitated by changes in the carbon dioxide and lactic acid contents of the blood are taken into account, the ingestion of fructose actually produced a greater increase in the respiratory quotient than did the ingestion of glucose. This is particularly true in the periods from 90 to 180 minutes. With galactose the average changes are almost the same as those found with glucose, although the agreement between the two subjects is not so good with galactose.

In general, the greatest increases in the respiratory quotient are after fructose ingestion. This implies that there is a more rapid combustion of carbohydrates after fructose than after glucose or galactose

ingestion. The respiratory quotients considered in table 4 are the total quotients. Some of them approach or equal unity, and if corrections are made for protein combustion in the calculations, the non-protein quotients in these cases will be over 1.00. This applies particularly to those after fructose and after galactose but rarely after glucose ingestion. Respiratory quotients over 1.00 are interpreted as indicative of formation of fat from carbohydrates. It would seem from the increases in the

TABLE 4

Net changes in respiratory quotients ascribable to sugar ingestion.

SUBJECT AND TYPE OF EXPT.	DATE (1936)	BASAL R. Q.	MINUTES AFTER DOSE [1]				
			0–45	45–90	90–135	135–180	180–240
H. C. W.							
Glucose	May 19	0.80	− 0.03	+ 0.09	+ 0.06	+ 0.11	+ 0.09
	June 1	0.75	+ 0.02	+ 0.09	+ 0.12	+ 0.07	+ 0.03
Fructose	May 14	0.84	− 0.01	+ 0.10	+ 0.15	+ 0.12	+ 0.05
	May 28 [2]	0.77	+ 0.03	+ 0.12	+ 0.12	+ 0.09	+ 0.02
Galactose	May 12	0.78	+ 0.02	+ 0.03	+ 0.12	+ 0.08	+ 0.08
	May 23	0.78	+ 0.02	+ 0.06	+ 0.07	+ 0.07	+ 0.08
W. C.							
Glucose	June 8	0.82	− 0.01	+ 0.08	+ 0.16	+ 0.11	+ 0.06
	June 19	0.87	− 0.04	+ 0.06	+ 0.10	+ 0.07	+ 0.06
Fructose	June 12 [3]	0.83	+ 0.05	+ 0.08	+ 0.07	+ 0.09	− 0.01
	June 24 [3]	0.85	+ 0.02	+ 0.12	+ 0.15	+ 0.02	+ 0.02
	June 26	0.81	− 0.01	+ 0.14	+ 0.16	+ 0.16	+ 0.05
	June 29	0.77	− 0.06	+ 0.01	+ 0.13	+ 0.10	+ 0.03
Galactose	June 10	0.83	+ 0.04	+ 0.12	+ 0.14	+ 0.11	+ 0.04
	June 22	0.83	− 0.03	+ 0.16	+ 0.13	+ 0.16	+ 0.04
	July 1	0.84	− 0.01	+ 0.10	+ 0.08	+ 0.11	+ 0.02

[1] 100 gm. of sugar unless otherwise noted.
[2] 50 gm. of sugar.
[3] 75 gm. of sugar.

respiratory quotient over basal that the order of possibility in this respect is fructose, galactose, and glucose. Therefore, these high respiratory quotients may result from the conversion of sugar to fat as well as from increased combustion.

SUMMARY

The effects of the ingestion of 50, 75, or 100 gm. of glucose, fructose, or galactose on the alveolar carbon dioxide, the blood lactic acid, and

the respiratory quotient were studied over a period of 4 hours with two normal men. In comparison with control experiments, the alveolar carbon dioxide showed a significant change (decrease) only in the galactose experiments. The blood lactic acid increased after all three sugars, the increase being most marked and lasting longest after fructose. The respiratory exchange measurements were corrected for the effects of these changes in blood lactic acid and alveolar carbon dioxide, and the resultant net increases in the respiratory quotients ascribable to the ingestion of the sugars were calculated. After glucose the maximum net increase in quotient occurred within 90 to 135 minutes, and there was no return to the pre-ingestion level in 4 hours. After galactose the net increases were about the same as after glucose. The greatest net increases in the respiratory quotient were produced by the ingestion of fructose. The differences in the rises in quotient indicate differences in the rapidity of combustion of the sugar or differences in the rate at which sugars may be transformed into fat.

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THE THIAMINE REQUIREMENT OF PIGS AS RELATED TO THE FAT CONTENT OF THE DIET [1]

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TWO FIGURES

(Received for publication August 19, 1943)

Data have been presented in a previous article by Van Etten, Ellis, and Madsen ('40) which indicate that young pigs of 1 to 3 months' age require 106 to 120 μg. of thiamine (vitamin B_1) per 100 gm. of carbohydrate and protein for normal growth. On a body-weight basis and for the diet used, these figures corresponded roughly to 37 μg. of thiamine per day per kilogram of body weight. This value, which corresponds to approximately 1.7 mg. per 100 pounds of body weight, is somewhat higher than the figure of 1 mg. given by Hughes ('41) who worked with pigs beyond the usual weaning age. The earlier study was based on the use of a diet which contained approximately 10% of fat. The results were further interpreted to indicate that the thiamine requirement of the pig is directly related to the carbohydrate-protein intake.

Such a relationship has been shown in the rat by Evans and Lepkovsky ('29) and in the dog by Arnold and Elvehjem ('39).

Accordingly, additional work was undertaken to establish the relationship of thiamine intake to that of dietary fat and to determine more definitely the thiamine requirements on the basis of carbohydrate-protein consumption. In addition, information was sought on the physiological and pathological response of pigs on different levels of thiamine including storage of the vitamin in the muscle tissue.

PROCEDURE

The role of dietary fat was studied at three levels, namely, approximately 2, 11, and 28%. The first one was selected as representing a

[1] Reported at the meeting of the American Society of Animal Production, November 28–30, 1941, and abstracted in the Journal of Animal Science, vol. 1, page 84.

[2] Acknowledgment is made of the assistance of C. H. Van Etten, resigned and G. S. Barkman in carrying out the details of the experimental work.

typical low level of fat such as is sometimes fed to pigs on the farm and at the same time supplying the necessary essential fat acids. The high level approximates the fat content in the total solids of sow's milk and the intermediate level, that of the mixture of grain feed and sow's milk consumed by suckling pigs from the age of 3 weeks to weaning. The diets were of the type used in the earlier work of Van Etten, Ellis and Madsen ('40) and were prepared from constituents which had been treated to remove or inactivate the thiamine without materially reducing the content of the other vitamin factors of the vitamin B complex. Rat assays on the diets have confirmed this premise. The composition of the diets is shown in table 1.

TABLE 1

Composition and comparative feeding levels of the diets.

ITEM	COMPOSITION AND FEEDING LEVEL OF DIETS		
	Low-fat	Medium-fat	High-fat
	%	%	%
Casein, extracted	22.3	25.0	30.3
Dried whey, SO_2 treated	10.7	12.0	14.5
Dried liver, SO_2 treated	6.7	7.5	9.1
Salt mixture	3.1	3.5	4.2
Agar	1.8	2.0	2.1
Lard	1.0	10.0	28.0
Dextrin	55.4	40.0	11.8
Total	100.0	100.0	100.0
Isocaloric feeding level [1]	4.5	4.0	3.3

[1] Daily feed based on percentage of live weight. The daily allowance in calories per kilogram of live weight was approximately 165.

Normal healthy pigs were removed from the sows at the age of 3 weeks and placed in individual cages provided with wire floors, which were located in a temperature-controlled laboratory room maintained at 77° ± 3°F. Their weights ranged from 3 to 6 kg. They were assigned at random to the three diets. A limited number designated as positive controls were also fed the medium-fat diet similar to that given in table 1 except that the casein, whey, and liver were not treated to remove or inactivate the thiamine. The pigs were fed the diets at levels calculated to provide an equal caloric intake and found by earlier work to provide a full feed to the majority of animals. The figures for the feeding levels are shown in table 1. When these pigs, which had been designated to receive thiamine therapy, showed characteristic signs of depletion, the supplemental feeding of thiamine was initiated.

These signs included failure of appetite, occasional vomiting, scouring, and cessation of growth. Records of body temperature and heart-beat along with feed consumption were also kept throughout the experiment.

Thiamine therapy was based on body weight but since feed allowance was also on the same basis, the two were actually correlated throughout the therapeutic period. Most of the pigs were placed on levels of thiamine which were judged to be at or near the estimated requirements for the different diets which would give normal growth and enable the calculation of the ratio of thiamine to carbohydrate and protein as desired. The levels actually employed are shown in table 2.

TABLE 2

Results of feeding tests on young pigs showing growth response in relation to thiamine intake on different levels of dietary fat.

DIET	THIAMINE LEVEL PER KILOGRAM OF BODY WEIGHT	NUMBER OF PIGS	DEPLETION PERIOD		THIAMINE THERAPY PERIOD			
			Average time[1]	Average daily gain	Average time	Average daily gain	Feed per kg. gain of body weight	Thiamine per 100 gm. protein and carbohydrate
	μg.		*days*	*kg.*	*days*	*kg.*	*kg.*	*μg.*
Low-fat	0	4	25	.11	..	..	..	..
	15	1	22	.04	31	.08	2.00	55
	25	3	25	.12	39	.27	1.58	86
	40	4	24	.16	42	.42	1.47	113
	50	4	24	.11	54	.42	1.55	141
Medium-fat	0	4	28	.11	..	..	..	..
	15	1	22	.05	35	.15	1.50	54
	25	4	31	.13	48	.32	1.50	101
	35	4	26	.14	52	.46	1.27	125
	40	1	28	.14	42	.40	1.44	148
	100	1	27	.09	48	.43	1.15	341
High-fat	0	3	33	.16	..	..	..	..
	15	4	33	.12	48	.33	1.29	88
	25	4	37	.17	45	.48	1.18	135

[1] Indicates time until initiation of therapy was considered necessary. Survival times of pigs receiving no therapy averaged 39 days for low-fat, 49 days for medium-fat, and 60 days for high-fat.

They range from 15 to 100 μg. of thiamine in the form of thiamine chloride hydrochloride daily per kilogram body weight. The test periods for the majority of animals ranged from 10 to 13 weeks for the total of depletion and therapy. The experimental work extended through four farrowing seasons from the spring of 1940 through the fall of 1941. Results on a total of forty-five pigs, including three positive controls, are available.

All animals were examined at autopsy for pathological changes. Histological studies were also made on selected tissues. Assays for thiamine content were made by the rat-growth method on samples of hams from pigs selected to represent the more critical levels of therapy.

The details of the method of assay were adapted from the procedure described by Kline, Hall and Morgan ('41). Instead of using autoclaved yeast and peanuts and sulfite-treated liver extract to supply the vitamin B factors other than thiamine, commercial dried whey and fresh hog liver were treated with a sodium sulphite solution and then dried. The rat diet consisted of 25 parts of extracted casein, 9 of lard, 20 of sugar, 20 of dextrin, 2 of agar, 3.5 of salt mixture, 0.3 of cod-liver oil, 0.7 of wheat-germ oil, 12 of sulphite-treated whey, and 7.5 (dry basis) of sulphite-treated liver.

The rats were depleted of their thiamine stores by feeding the basal diet without added supplements and then were assigned in groups to the test materials. Two and sometimes three groups were fed graded levels of thiamine chloride hydrochloride three times a week. The samples of ham tissue were weighed out and fed on the same days as the controls on thiamine. The test period lasted 4 weeks and all animals were confined in individual cages. The thiamine content of the ham samples was obtained by comparison to the reference curves based on the growth of the animals fed thiamine chloride hydrochloride.

RESULTS

The feeding of the thiamine-deficient diets resulted in essentially the same type of symptoms as those recorded in the earlier work of Van Etten Ellis and Madsen ('40). Failing appetite usually was the first sign of thiamine deprivation. There was a direct relation to level of dietary fat. On the low-fat diet the average number of days for loss of appetite to appear was 21, while on the medium and the high levels it was 25 and 31 days, respectively. Although vomiting of food was observed it did not occur in many cases. On the other hand, scouring was much more common.

Retardation of growth followed closely the failure in appetite. In table 2 are recorded the average days on test during the depletion period. The time required for unmistakable evidence of thiamine deficiency to appear generally followed within a few days the periods already given for loss of appetite and averaged 25 days for the low-fat, 28 for the medium-fat, and 33 for the high-fat diet. The average survival time for the pigs that received no thiamine was 39, 49 and 60 days, respectively, for the low-, the medium-, and the high-fat groups.

The negative control animals which were continued on the basal diet with no thiamine added were either allowed to die from the effects of thiamine deficiency or were sacrificed when in a semi-moribund condition. Inspection of the daily heart-rate and semi-weekly rectal temperature records on these animals gives further information on the course and rate of depletion of animals when fed thiamine-deficient diets of varying fat content.

Individual data for representative litter-mate negative control pigs, together with response of a deficient animal to therapy in the spring series of 1941 are shown in figures 1 and 2.

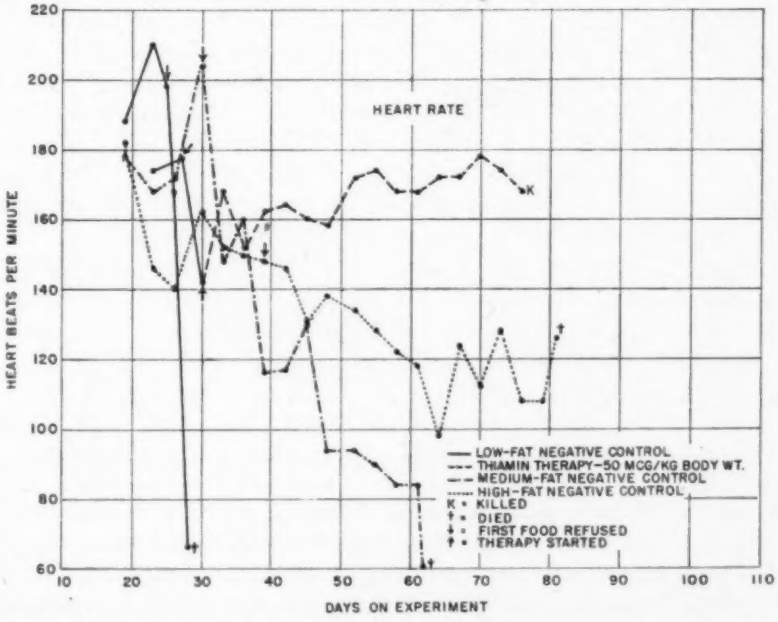

Fig. 1 The heart rates of typical individual pigs on different dietary treatments.

In figure 1 the plotted points represent the heart rate obtained by averaging values on three separate days, except in the case of the last value recorded which is the last observation before the animal died or was sacrificed.

It is obvious that slowing of the heart rate is a prominent symptom of thiamine deficiency. This became evident in the animals at about the same time that the first refusal of food was noted. Thereafter the decline in heart rate was rapid as long as no thiamine was given.

Changes in heart rate were usually accompanied by changes in rhythm and intensity of the beat. The heart of the animal on the low-fat diet failed rapidly. The animal on the medium-fat diet lived longer and had a slower rate of decrease. Similarly, the animal receiving the high-fat ration also had a gradual decrease in heart rate but the beat apparently became more variable toward the end of the survival period and did not reach as slow a rate as found for the lower levels of fat intake. In contrast the heart rate of the animal on the low-fat ration which was given 50 μg. of thiamine per kilogram of body weight after partial depletion, rapidly returned to normal.

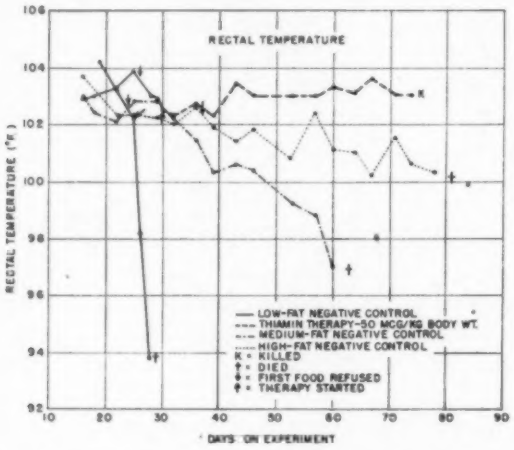

Fig. 2. The rectal temperatures of typical individual pigs on different treatments.

A similar picture is recorded in figure 2 which is a semi-weekly record of rectal temperatures taken on the same litter-mate negative control and thiamine-treated animals as shown in figure 1. The temperature of the animal on the low-fat ration dropped rapidly, the animal on the medium-fat dropped more slowly, and still more slowly for the animal on the high-fat. The temperature of the animal on the high-fat was more variable, yet higher, than in the case of the animals receiving the lower amounts of fat. The animal on the low-fat diet responded very shortly with an increase in body temperature after thiamine therpay was started.

The usual findings noted at autopsy in animals dying of thiamine deficiency were extreme emaciation, flabby heart muscle, congested liver, and occasionally free fluid in the abdominal, thoracic, and pericardial cavities.

In contrast to these negative control animals, a number of pigs fed as positive controls with the thiamine supplied through the untreated whey, liver, and casein, maintained a rate of growth approximating 0.4 of a kilogram per day over a period of 10 weeks. Furthermore, the symptoms of poor appetite, emaciation, slowing of the heart rate, and drop in temperature were absent.

Microscopical examination of a number of tissues from the negative control pigs which were usually in advanced stages of thiamine deficiency, failed to demonstrate any consistent lesions of the sciatic nerve, spinal cord, pancreas, thyroid, kidney, spleen, or adrenal glands. Various stages of myocardial degeneration were confirmed as previously reported by Van Etten, Ellis and Madsen ('40) and by Follis et al. ('43) and these changes are probably related to the slower heart rate and abnormalities of conduction previously noted by Madsen ('42) and described in this study. The heart lesions tended to be more severe in pigs fed the low- and medium-fat rations without thiamine supplement than in the high-fat negative controls. Small, apparently healed areas consisting of atrophy or complete disappearance of cardiac muscle fibers often accompanied by an infiltration of mononuclear cells were seen in the hearts from some of the pigs that had been partially depleted of thiamine and then fed adequate levels of this vitamin. This suggests that injury to the heart may occur along with the other early criteria of deficiency such as inappetence and slowing of the growth rate or less in weight. Varying degrees of atrophy of the gastric glands in the pyloric region were noted in most of the thiamine-deficient pigs. The most severe case occurred in a negative control pig on the high-fat ration, that died after 82 days on the deficient ration.

THIAMINE THERAPY

In most cases, the animals depleted on the thiamine-low diets responded immediately to the feeding of thiamine. The appetites returned to normal, vomiting usually ceased, the body temperatures returned to normal, and resumption in growth was noted within a week. There was a striking and characteristic difference in the performance of the animals fed the same amount of thiamine on the body weight basis but on different levels of dietary fat. These differences are shown in table 2. For example, 25 μg. of thiamine were given to pigs on all three diets. The average daily gains increased from 0.27 kg. on the low-fat diet to 0.48 kg. on the high-fat. To produce approximately the equal of the gain noted on the high-fat diet required the feeding of at least 40 μg. of thiamine on the low-fat and 35 on the medium-fat diet. At these levels the thiamine consumption per 100 gm. of protein

and carbohydrate was not greatly different since it fell within the range of 125 to 141 μg. These figures are somewhat higher than the range of 106 to 120 μg. previously suggested and probably allow a somewhat greater margin above the bare requirement for normal growth. It is noteworthy that the daily gains made by the pigs on the higher thiamine levels were approximately 1 pound, a rate much above the usual average for pigs of 6 to 13 weeks in age. The data for economy of feed utilization shown in table 2 also reveal some unusual figures which are probably attributable to the confinement of the pigs in a room maintained at a uniform temperature. At the time of slaughter,

TABLE 3

Electrocardiograph readings on pigs with and without thiamine present in their ration.

PIG NUMBER AND DIET TREATMENT	HEART RATE	P-R INTERVAL	Q-T INTERVAL	Q-T INTERVAL CORRECTED FOR CYCLE LENGTH=K	ALGEBRAIC SUM OF QRS IN LEAD I	ALGEBRAIC SUM OF QRS IN LEAD II	ALGEBRAIC SUM OF QRS IN LEAD III
	beats per min.	*sec.*	*sec.*	*K.*	*millivolts*	*millivolts*	*millivolts*
A-low fat, no thiamine	68	0.10	0.34	0.37	+ .15	+ .02	0.0
B-medium fat, no thiamine	85	0.14	0.35	0.40	− .29	+ .13	+ 0.64
C-medium fat, 25 μg. thiamine	125	0.10	0.27	0.39	+ .14	+ .63	+ .60
D-positive control thiamine present ...	138	0.10	0.21	0.34	+ .38	+ 1.10	+ .47

the pigs which had received levels of thiamine adequate for growth were fully as fat if not fatter than the average pig in the herd from which these were obtained.

Through the courtesy of Dr. H. O. Calvery and Dr. Bert J. Vos, Jr., of the Food and Drug Administration, electrocardiograms were secured on four pigs fed during the spring of 1940. The data are given in table 3. Two of the pigs (designated as A and B) had been on low-thiamine diets for 50 days, had been losing weight for approximately 3 weeks and generally showed the usual advanced symptoms of thiamine deficiency. Pig C, at the time the electracardiograms were made, had been receiving 25 μg. of thiamine per kilogram of body weight for approximately 2 weeks following a 4-week depletion period, and had fully recovered his appetite and general state of well-being. The fourth pig had been fed for about 50 days on the positive-control diet with adequate amounts of thiamine present. All four animals had been handled daily and were, therefore, good subjects for study. Very little

difficulty was encountered in making the electrocardiograms on the two thiamine-deficient pigs. Pig D, the positive control, was the most active and the most difficult to restrain while the readings were being made.

The heart rate as shown in table 3 was unmistakably slower in the two pigs which had been deprived of thiamine than in the two with the vitamin present in their diet. Pig B had a right axis deviation as shown by the negative value of the algebraic sum of QRS in lead 1. This finding is frequently associated with hypertrophy of the right ventricle.

TABLE 4

Influence of level of thiamine fed to pigs on the content of this substance in ham muscle.

LEVEL OF THIAMINE FEEDING (MICROGRAMS PER KILOGRAM OF LIVE WEIGHT OF PIG)	MICROGRAMS OF THIAMINE PER 100 GRAMS OF HAM MUSCLE IN PIGS FED DIET DESIGNATED		
	Low-fat	Medium-fat	High-fat
0	..	Trace	Trace
15	..	..	4 [1]
25	19	24 [1]	35 [1]
35	..	33 [2]	..
40	17	60	..
50	39 [1]	..	..
100	..	168	..

[1] Represents average of two pigs.

[2] Represents average of three pigs; all other data are for one pig each.

The K values, as indices of the speed of ventricular contraction, were slightly higher in pigs A and B than in pig D. The high value for pig C, which had been receiving thiamine for approximately 2 weeks, however, suggests that the differences may be due to random variability unless the therapy period of pig C had been insufficient to allow for correction of the abnormality.

These data are believed to be fully in line with other evidences of heart damage, not only in pigs but in other animals.

The assays for thiamine content of the lean tissues from the hams of a number of the experimental pigs are shown in table 4. Comparable assays on two herd pigs that had been fed an ordinary diet of corn and supplements and slaughtered at the same ages as the experimental pigs, gave values of 785 and 795 μg. of thiamine per 100 gm. of fresh tissue. These values are in turn somewhat lower than found in older pigs weighing 225 pounds, where the values range from 690 to 1520. Compared to these values, the experimental pigs did not store appreciable amounts of thiamine although the figures in table 4 show a gradual but consistent increase in thiamine content of meat tissue with increase in dietary fat and thiamine intake. It is evident that the thia-

mine requirements for maintenance and growth are met before appreciable storage takes place and furthermore that the extent of storage is proportionate to the level of thiamine. The low figures for storage of thiamine are in harmony with those of Hughes ('41). That even commercial pork may vary in thiamine content through wide limits has been shown by Waisman and Elvehjem ('41) who have reported values on pork hams ranging from 910 to 1900 μg. per 100 gm. of fresh tissue.

SUMMARY

The thiamine requirements of young pigs have been studied on three diets containing approximately 2, 11, and 28% of fat. As indicated by failure in appetite and cessation of growth, the animals on the low level of fat showed evidence of thiamine depletion on the average in 25 days, those on the medium level in 28 days, and on the high level in 33 days. Lack of thiamine resulted in marked weakening of the heart, decrease in body temperature, emaciation, and other changes.

When thiamine was fed to pigs depleted of their stores of this substance, the response in appetite, growth, and general health was usually prompt and striking. Intermediate levels of thiamine produced the greatest response in the pigs fed the high-fat diet, followed in order by those on the intermediate and the low-fat. It was found that the level of thiamine required to produce a maximum rate of growth and otherwise maintain the pigs in good health fell within the range of 125 to 141 μg. per 100 gm. of carbohydrate and protein. These levels of thiamine, however, were insufficient to promote the storage of normal amounts in the meat tissue such as is found in commercial pork cuts.

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INTERRELATION OF METHIONINE, CHOLINE, BETAINE AND ARSENOCHOLINE IN THE CHICK

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(Received for publication September 13, 1943)

In a preceding report (Grau and Almquist, '43) we have shown that the choline-deficient chick can utilize homocystine in lieu of cystine, but not in lieu of methionine. Results of additional studies, which include the effect of methionine, betaine and arsenocholine as substitutes for choline, may now be presented. The general procedure and the basal diet have been described in detail (Grau and Almquist, '43). Briefly, choline-depleted chicks were fed a synthetic-type diet in which the protein source was an isolated soybean protein of known methionine and cystine contents. The three or four chicks used in each group were carefully selected with respect to weight, rate of gain in weight, and degree of choline depletion. The experiments were carried on for 1 week, during which time the chicks were weighed daily.

Some of the data now reported were obtained simultaneously with, and all are directly comparable to, those in the preceding report. For convenience in making comparisons, the diets have been numbered in a continuous series with those of the first paper, from which report pertinent data have been summarized in table 1. Percentage daily rate of gain will be referred to simply as gain.

A preliminary communication (Almquist and Grau, '43) has been made on the growth-promoting effect of betaine (glycine betaine). It was reported that under certain conditions betaine may promote growth as effectively as choline or methionine. Earlier reports of a growth-promoting effect of betaine in chick diets have been made (Jukes, '40; Jukes and Welch, '42; McGinnis, Norris and Heuser, '42).

RESULTS

The extended studies with betaine (table 2) demonstrate a growth-promoting action which becomes conspicuous under conditions of choline and methionine deficiency. Betaine alone caused no appreciable increase in gain (compare diets 1 and 24). Insofar as the added sulfur

amino acids consisted of cystine or homocystine or both, the addition of betaine allowed gains of approximately 4.0% per day. The increases in gain due to betaine addition were approximately 2.0 throughout (compare diets 24 and 25, 5 and 26, 6 and 28, 10 and 29, 11 and 30). In the presence of methionine, however, the addition of betaine led to very little further increase in rate above 4.0 (compare diets 12 and 32). All of these diets, evidently, lacked some growth-promoting substance, the effect of which was not appreciably provided even by excesses of betaine or methionine, but which was provided by the diets containing choline (i. e., diets 19, 21, 22, 23 and 41).

TABLE 1

Summary of pertinent data from the report of Grau and Almquist ('43).

DIET NUMBER	SUPPLEMENTS ADDED TO THE BASAL DIET [1]				TOTAL MILLIMOLS SULFUR AMINO ACIDS ADDED PER 100 GM. DIET	AVERAGE % GAIN PER DAY
	1-Cystine	dl-Homocystine	dl-Methionine	Choline chloride		
1	...	...	...	...	...	1.2
2	0.20	...	...	...	1.67	1.8
3	0.40	...	...	...	3.34	1.9
5	...	0.25	...	...	1.72	1.8
6	...	0.45	...	...	3.36	1.5
7	...	...	0.25	...	1.68	3.7
8	...	...	0.50	...	3.36	4.2
9	...	...	1.00	...	6.72	4.3
10	0.20	0.23	...	...	3.39	2.0
11	0.20	0.45	...	...	5.03	1.6
12	0.20	...	0.50	...	5.03	4.4
19	0.20	0.45	...	0.20	5.03	6.4
21	0.20	...	0.50	0.05	5.03	6.0
22	0.20	...	0.50	0.20	5.03	6.0
23	0.20	...	0.70	0.20	6.37	6.3

[1] Expressed in per cent added to diet.

Arsenocholine additions, as in the case of betaine and methionine additions, without choline, led to similar gains, approximately 4.0, by the chicks on diets containing added cystine and homocystine (diets 34, 35, 36 and 37). An important difference from the betaine results was observed in the case of diets 38 and 42, where the substitution of arsenocholine for betaine in the presence of added methionine led to definitely better growth. More striking, however, were the results with the combinations of betaine and arsenocholine, which yielded optimal gains (compare diets 37 and 39; also diets 32 and 44).

TABLE 2

The relation of methionine, betaine and arsenocholine to the choline requirement of the chick.

DIET NUMBER	SUPPLEMENTS ADDED TO THE BASAL DIET [1]						TOTAL MILLIMOLS SULFUR AMINO ACIDS ADDED PER 100 GM. DIET	NUMBER OF GROUPS	AVERAGE % GAIN PER DAY
	l-Cystine	dl-Homocystine	dl-Methionine	Betaine hydrochloride	Arsenocholine chloride	Choline chloride			
24	...	...	...	0.22	...	...	...	3	1.4
25	0.20	...	...	0.22	...	...	1.67	3	3.4
26	...	0.23	...	0.22	...	...	1.72	1	4.1
27	...	0.23	...	0.30	...	...	1.72	1	4.0
28	...	0.45	...	0.22	...	...	3.36	1	3.6
29	0.20	0.23	...	0.30	...	...	3.39	1	4.1
30	0.20	0.45	...	0.22	...	...	5.03	2	4.1
31	0.20	0.45	...	0.50	...	...	5.03	1	4.0
32	0.20	...	0.50	0.22	...	...	5.03	2	4.6
33	0.20	...	0.70	0.22	...	...	6.37	1	4.8
34	0.20	...	...	...	0.29	...	1.67	1	3.8
35	0.40	...	...	...	0.29	...	3.34	1	3.8
36	0.20	0.23	...	...	0.30	...	3.39	1	3.8
37	0.20	0.45	...	...	0.29	...	5.03	1	4.2
38	0.20	...	0.50	...	0.29	...	5.03	1	5.7
39	0.20	0.45	...	0.22	0.29	...	5.03	1	6.6
40	0.20	...	0.50	0.22	...	0.03	5.03	1	5.3
41	0.20	...	0.50	0.22	...	0.05	5.03	1	6.0
42	0.20	...	0.75	...	0.29	...	6.42	1	5.8
43	0.20	...	0.50	0.22	0.07	...	5.03	1	5.3
44	0.20	...	0.50	0.22	0.14	...	5.03	1	6.3

[1] Expressed in percent added to diet.

DISCUSSION

The growth-effect of betaine is not readily accounted for on a basis of facilitating choline synthesis in the chick. This is indicated by the fact that betaine is not appreciably effective in the prevention of choline-deficiency perosis (Jukes, '40; Jukes and Welch, '42).[1] Other evidence showing a lack of ready choline synthesis in the chick, even in the presence of an excess of methionine or of methionine plus ethanolamine, is available (Jukes, '41; Record and Bethke, 42).[2]

It is noteworthy that the increases in gain upon addition of betaine were but slightly better with homocystine than with cystine (compare diets 2 and 25, increase in gain 1.6%/day; with diets 5 and 26, increase 2.3; diets 6 and 28, increase 2.1; diets 11 and 30, increase 2.5). An excess of betaine (diet 31) or of homocystine (diets 30 and 31), in relation to gain observed, did not seem to increase the gain further. That incomplete methylation of homocystine is not the full explanation for the lack of optimal gain is shown by the fact that replacement of the homocystine by methionine (diet 32) permitted only a slightly better gain, which was still far below the optimum.

That methionine exerts an effect similar to that of betaine is indicated by the results with diets 7, 8 and 9, as compared with 2. In the case of diets 7, 8 and 9, practically all sulfur amino acids were in the form of methionine, some of which must have been converted in vivo to cystine. These diets could hardly have been expected to be so markedly superior to diet 2, which had 0.20% cystine added to it, and also contained 0.30% methionine present in the basal diet. Lack of certain choline functions is indicated here also, for the gains with any level of methionine did not appreciably exceed 4.0% per day. Furthermore, betaine and methionine fed in combination did not provide the missing effects of choline (diets 32 and 33).

Since the conception that betaine may serve as a precursor of choline in the chick appears either untenable or inadequate, on the basis of

[1] McGinnis, Norris and Heuser ('42), using a diet differing greatly from that of Jukes, reported a positive antiperotic action of betaine and indications that betaine and ethanolamine fed jointly were somewhat more effective than betaine alone. In view of this apparent controversy we repeated the studies of Jukes with choline and betaine and completely confirmed his original findings. The combination of betaine and ethanolamine was also found to be ineffective in preventing perosis. Professor Norris has indicated in private correspondence that antiperotic effects with betaine were not obtained with still another type of diet and that an explanation of the results first reported is being sought.

[2] We may also call attention to further data confirming the above reports on the lack of effective antiperotic action by betaine, methionine or their combinations with ethanolamine, provided in the Nutrition News Letter no. 11 of the Archer-Daniels-Midland Co., Minneapolis, Minnesota, under the date of May 28, 1942.

present knowledge, it is difficult to explain the effects of betaine except by assuming that it is physiologically important to the chick. It is possible that betaine may even be formed in the chick from choline or methionine. Whatever may be the explanation, all three of these substances are capable of exerting a similar growth-promoting effect which we shall designate as B (betaine effect). This effect B is undoubtedly the cause for earlier results indicating that betaine gave growth effects which were either small or of doubtful significance (Jukes, '40; Jukes and Welch, '42; McGinnis, Norris and Heuser, '42).

In contrast to betaine, arsenocholine has been reported to be strongly antiperotic and growth-promoting in the fowl (Jukes, '40; Jukes and Welch, '42). It is unable to promote appreciable methylation of homocystine in the chick (Almquist and Jukes, '43) or in the rat (Welch, '41; Moyer and du Vigneaud, '42). Arsenocholine enters into the synthesis of phospholipids as a structural component replacing choline (Welch and Landau, '42), hence it may be particularly useful to the choline-deficient chick, in which choline synthesis appears, at best, to be a very inadequate process.

In the case of diets containing arsenocholine without betaine or added methionine, distinct but limited increases in gain (approximately 2.0% per day) were observed (compare diets 2 and 34, 3 and 35, 10 and 36, 11 and 37). Arsenocholine in the presence of added methionine caused an appreciably greater increase in gain (diet 38) than did betaine in a similar diet (32) (compare also diets 42 and 33). Most striking of all was the optimal growth obtained with the combination of betaine and arsenocholine (diets 39 and 44). It is evident that arsenocholine imparts certain additional growth-promoting influences which we shall designate as A (arsenocholine effect).

Our present concept is that arsenocholine provides effect A but very little effect B; hence growth with arsenocholine is limited by the lack of effect B. The converse of this seems true with betaine and methionine. In the presence of betaine or methionine or both, the effect of arsenocholine is additive, and the gains are similar to those obtained with choline, which provides both effects A and B. Each effect is undoubtedly multiple in nature.

Data showing a normal retention of bound choline in the liver and muscle tissues of severely perotic chicks reared on choline-deficient diets have been kindly made available by Dr. Jukes.[3] His comment is of interest: "... choline is a 'building stone' and if it is deficient less tissue is formed." It seems highly probable that bound choline in tissues con-

[3] Private correspondence.

stitutes the primary demand on choline; hence substances which will spare or simulate other functions of choline in a choline-depleted animal produce pronounced effects. Such sparing action may be included in both effects A and B. One must also recognize the possibility that tissue-bound choline may be able to promote methylation processes and growth may then ensue if further functions of choline are provided by suitable substitutes.

In the presence of an ample source of effect B (diets 21 and 41), 0.05% of choline chloride sufficed for approximately optimal gain, which suggests that this amount of choline was sufficient to provide effect A. It seems probable that the level of arsenocholine used in these studies was also ample to provide effect A, except in the case of diet 43. On a molar basis, arsenocholine appears to be approximately one half as active as choline in providing effect A.

The minimum amount of methionine required in the diet of the chick for optimal gain is approximately 0.55%, as pointed out in the preceding report. In the case of diets such as 19, in which optimal gain was obtained with homocystine and choline additions, approximately half of the homocystine must have been methylated to provide an adequate level of total methionine (0.30 in the basal diet plus 0.25 by methylation of homocystine). The remainder of the homocystine converted to cystine would then complete the sulfur amino acid requirement of the chick. In the case of diet 39, which contained betaine and arsenocholine in place of choline, it is apparent that a similar conversion of the homocystine to methionine and cystine must have taken place. This implies that appreciable methylating power may be ascribed to betaine (or to tissue-bound choline) in the choline-depleted chick.

When the criterion for transmethylation is growth of animals on a choline-deficient, homocystine-supplemented diet, it appears possible that the methylating power of compounds may be underestimated by virtue of a deficiency of certain of the growth-promoting influences of choline. In such studies the inclusion in the diet of some relatively non-methylating substance such as arsenocholine (to provide effect A) would seem advisable.

The results of these studies show that the practical dietary choline requirements of animals may be modified by the adequacy or deficiency of betaine and methionine. Betaine is of widespread occurrence in feedstuffs. The choline-like activities of practical feedstuffs are, in all probability, the resultant of the combined effects of choline, betaine and methionine. The present work furnishes no clear information on a methionine-sparing action of choline and betaine. This will require

further study. However, a choline-sparing action on the part of methionine and betaine is distinctly indicated.

SUMMARY

1. Betaine (glycine betaine) and methionine are capable of assuming a certain portion of the functions of choline in the chick.

2. Arsenocholine is capable of assuming the remainder of the choline functions.

3. The effects of betaine (or methionine) and arsenocholine are additive, and, together, are practically a complete substitute for choline under the conditions of these experiments.

ACKNOWLEDGMENT

These studies were facilitated by research grants from Swift and Co., and the Nutrition Foundation Inc. Arsenocholine was kindly made available by Dr. A. D. Welch of Sharp and Dohme, Inc., and the soybean protein by J. L. Gabby of the Glidden Co. The authors are indebted to Dr. T. H. Jukes, Prof. C. L. A. Schmidt and Dr. A. D. Welch for helpful suggestions and criticism of the manuscript.

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THE MINIMUM ASCORBIC ACID NEED OF ADULTS[1]

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FIVE FIGURES

(Received for publication September 29, 1943)

In spite of the extensive literature on human nutrition there is still need for more data on minimal safe levels of intake of the essential food factors. Early in 1942 we conceived the idea of using male prisoners as subjects for a study of some of the basic nutritional requirements of healthy adults. The handicap of a 55-mile distance separating the Wisconsin State Prison and our laboratory was largely compensated for by the cordial assistance given us by the warden and medical staff of the prison hospital. Subjects were selected from volunteers after the project had been explained to the entire inmate population of more than 1100 over the prison radio. The group was more than ordinarily homogeneous in that the men were on a controlled and regular regime. They were regularly available to our convenience for examination and tests; their cooperation was excellent. Information concerning diet, habits, history and physical condition was at hand.

A preliminary study was made on a group of fifty-six men whose records showed no major illnesses for at least a year. Nearly all were of military age and had a fair to good I.Q. rating. All had been in prison for at least 1 year with sentences not to be completed for a year or more. Before the preliminary survey was completed it became obvious that most of the examination records indicated the presence of oral disease. We found almost universally extremely low plasma ascorbic acid values. Therefore, in May, 1942 a more detailed investigation of the Vitamin C factor was begun. This paper reports the findings in our ascorbic acid studies.

PROCEDURE

Intensive study, which included 778 determinations of plasma ascorbic acid, was made of seventy-one subjects. We were furnished menu sheets

[1] This study has been supported by special grants from the Wisconsin Alumni Research Foundation and from the Nutrition Foundation to one of us. (E.L.S.)

[2] Major, M.C., U.S.A., 44th General Hospital.

listing every item of food for each meal served to all inmates from March, 1942 to July, 1943. Records were kept of all fruits eaten in addition to regular diet.

At the first examination each subject's gums were inspected and the observations recorded. Tourniquet tests were done but were obviously unsatisfactory, and consequently were not repeated. Blood samples were obtained at least 3 hours after breakfast. The amounts of ascorbic acid in this meal were so small that the blood values would not have been materially altered in any case. Mindlin and Butler's ('38) photocolorimetric method was used without the KCN as an anti-oxidant. Scrupulous precautions were taken to guard against hemolysis when the blood was drawn, since this is known to cause loss of ascorbic acid by oxidation, catalyzed by the red cell contents. Blood specimens were iced immediately and brought to the laboratory on ice within 3 hours after withdrawal.

Vitamin C in drawn blood has been found in our laboratory and elsewhere to be stable within practical limits for many hours providing, as was our practice: (1) that the plasma is not separated from the cells when the sample must stand; (2) that there is no hemolysis; and especially (3) that, in addition, the blood is kept cold when it cannot be sterile. It has also been known for a long time that the vitamin is stabilized in the metaphosphoric acid filtrate.

To test the safety of carrying whole blood on ice for delayed analysis, twelve samples were drawn in such quantity that part could be taken to our laboratory in the usual way, and another portion centrifuged at the prison and the protein-free plasma filtrate made promptly for analysis as a duplicate after return from the prison. The results in these duplicate analyses agreed within the margin of error of the method. Differences in all cases were less than 0.1 mg. % which is the maximum variation between aliquots from the same blood in our use of the Mindlin-Butler method. Two showed no difference, and eight were less than 0.04 mg. % apart, with some figures higher and others lower in the filtrates promptly made. Keeping qualities by either way of handling were therefore judged to be satisfactory, so that we pursued the far simpler method of delaying the whole analysis until our return to the laboratory.

A further partial check on the effect of delayed analysis as such was made by taking samples from three of the authors while at the prison, and comparing these results with those of promptly analyzed samples obtained on the same individuals at the same time on the following day while they were in the laboratory. The repeated analyses

agreed within 0.04, 0.09, and 0.01 mg. %. Although the promptly analyzed sample in each case was the higher, the difference was certainly well within the expected error of the method in routine use.

Before supplements of ascorbic acid were given, each man had two or more control plasma vitamin C values recorded. Blood specimens were obtained usually once a week during the first 8 months of the study, thereafter less often. Four or more untreated subjects were included with the supplemented ones to serve as controls on the possible effects of season or changes in the institutional diet (fig. 1). An inspection

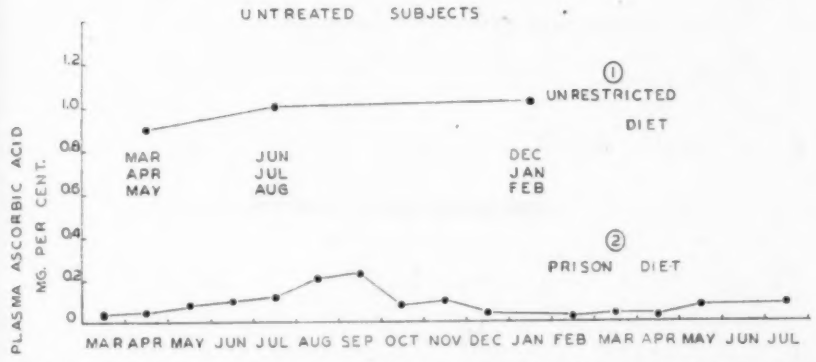

Fig. 1 Comparison of (1) the seasonal (spring, summer, winter) averages of single plasma ascorbic acid values on a miscellaneous group of thirty-one medical and home economics students and (2) the monthly averages of untreated subjects on prison diet, with or without occasional fruit intake. Subjects were excluded who reported daily use of vitamin preparations or fruit equivalent to one orange daily.

of the gums was made each time. Note was made of extra fruit eaten or of any change in health status. Of the seventy-one men, on first examination 77% had a plasma ascorbic acid level below 0.2 mg. %, 20% had between 0.2 and 0.6 mg. %, while only two (3%) had over 0.6 mg. %.

RESULTS

In May, 1942, twelve men were started on 25 mg. of synthetic ascorbic acid daily. The prison hospital staff supervised the daily distribution of tablets and made certain that each man was actually taking his dose. Control plasma values for this group were all below 0.2 mg. %. After 5 weeks the rise was insignificant. Accordingly, the daily dose was increased to 50 mg., which in 2 weeks' time definitely elevated the plasma level (fig. 2).

Other groups were started meanwhile on daily supplements of 50 mg. and 75 mg. Much individual variation in response to the same dose was noted. There were forty-five men who were available for the 6 to 14 months' period of supplementation necessary to establish and maintain a satisfactory plasma ascorbic acid level for several weeks

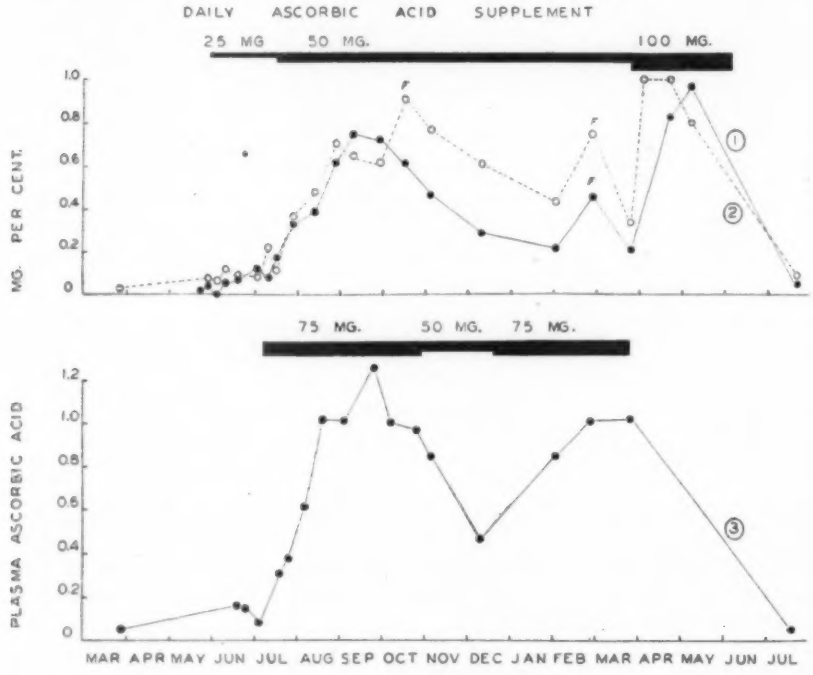

Fig. 2 Typical individual curves of blood response to different amounts of supplement.

Curve (1), subject no. 50, age 22, prison inmate 3 years.
Curve (2), subject no. 8, age 32, prison inmate 11 years.
Curve (3), subject no. 9, age 25, prison inmate 2 years.
(F) indicates recent intake of citrus fruit.

and to show satisfactory improvement of the gum conditions. Unexpected causes such as parole or removal to one of the prison farms interrupted the study of a few others. The rest studied were untreated control subjects. Among the forty-five cases, there were three subjects whose plasma response was unsatisfactory until the daily supplement was increased to 100 mg. (fig. 3). Observations of the gums of these men while taking 50 and 75 mg. revealed a lack of the usual improvement noted in the others receiving the same amount of ascorbic acid. Further

investigation proved all three to have a history of chronic nose and throat infections, and in two of the men severe infection was also present in the gums. Among the other few who complained of frequent colds, none exhibited definite signs of such chronicity. There were other subjects whose gums appeared as severely affected as (or more so than) those of the three refractory subjects, but as we did not attempt any bacteriological study of the infecting organisms, it is difficult to say how much bearing the type of organism may have had on the question.

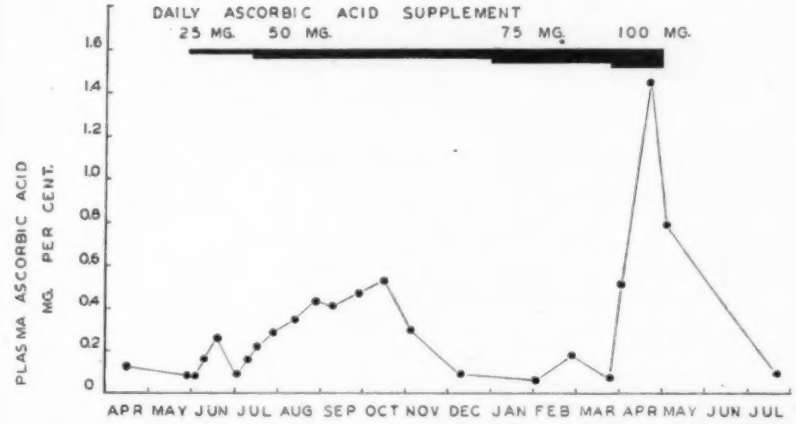

Fig. 3 Unsatisfactory blood response to supplements of 75 mg. or less. Subject no. 26, age 34, prison inmate 9 years, with a history and physical signs of chronic nose and throat infection; also advanced oral disease ("pyorrhea" cause of extraction of six lower front teeth).

Throughout August and September plasma ascorbic acid of the majority of subjects on 50 mg. supplements was maintained between 0.7 and 1.0 mg. % (fig. 2). This was the season in which a few raw vegetables such as onions and cabbages were on the menu. There may also have been an increase in the vitamin C content of the potatoes at the time, and in addition, more fruit was reported eaten as extras. The incidence of infections such as colds, was lower. Through the other months 50 mg. of supplement was inadequate (fig. 2). A slight seasonal rise for August and September may be seen on the curve (fig. 1) of the untreated controls.

A few subjects were carried through the winter on 50, 75, or 100 mg. of ascorbic acid daily. Upon withdrawal of supplements, a group of eighteen were given placebos. Typical results are illustrated (fig. 4).

Then, early in May, 1943, all supplements and placebos were withdrawn. The men were notified of the end of restrictions on extra fruit and vitamin pills. A last check was made late in July to determine the status of as many as possible. Thirty-four men were seen at this time. Plasma ascorbic acid levels of 28 (82%) were again below 0.2 mg. %; none were higher than 0.5 mg. %.

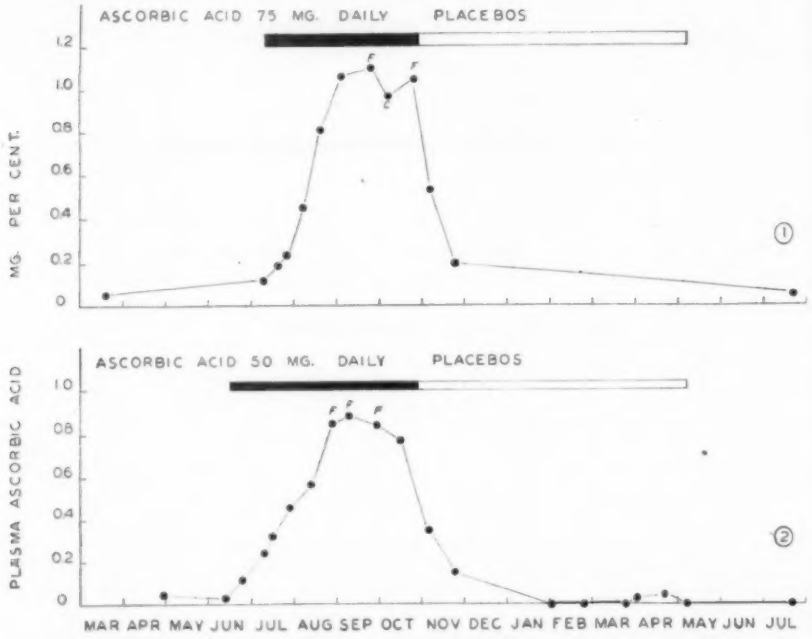

Fig. 4 Effect on plasma ascorbic acid of placebo substitution for supplements.

Curve (1), subject no. 3, age 27, prison inmate 6 years.
Curve (2), subject no. 41, age 31, prison inmate 9 years.
(F) indicates recent intake of citrus fruit.
(C) indicates recent nose and throat infection.

DISEASED GUMS

The most striking observation of the entire study was the relationship between the plasma ascorbic acid level and the health of the gums. Figure 5 illustrates a typical case in which are shown blood levels and brief notes of gum conditions made in corresponding months. Notes on the gums were made at the time of blood sampling and recorded before plasma values were known. One of us (E.D.K.) made all of the regularly recorded gum examinations. Three independent observations were

made by another (E.L.S.), two by E.S.G., and on another occasion we are pleased to acknowledge the opinions of Dr. T. A. Hardgrove, who has given considerable attention to the possible connection between dietary deficiency and gum conditions. Substantial agreement was noted by all four observers. We recognize, of course, that there are cases of gum disease which cannot be correlated with a history of low fruit intake or low plasma ascorbic acid values, and which do not respond to treatment with large doses of ascorbic acid.

The preliminary survey inspection revealed one subject whose gums appeared normal and free from disease. His plasma ascorbic acid reading was 0.95, the highest figure encountered in any untreated

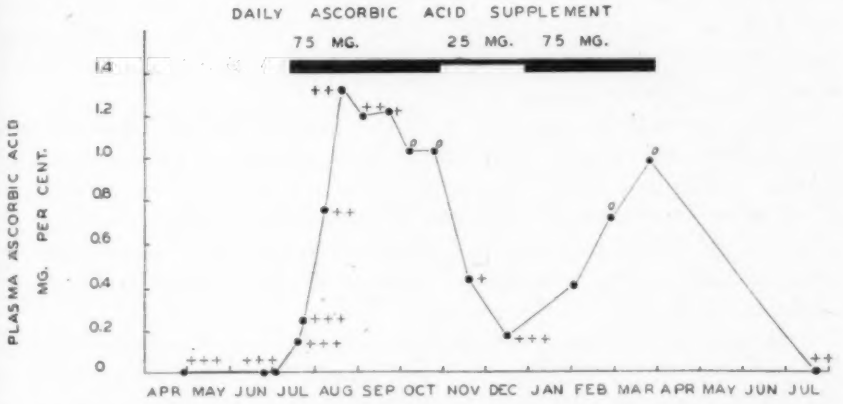

Fig. 5 Subject no. 39, age 25, prison inmate 2 years. Plasma ascorbic acid curve with gum changes indicated as follows:

Moderate to severe oral disease +++; slight to moderate ++; slight +; no disease 0.

4-30-42 — Marginal infection and recession — lower anterior, pallor with reddened and a few cyanotic areas, some edema, bleeding.

6-24, 7-1, 7-17 — No change.

7-22 — Color slightly improved.

8-5 — Color improved, no cyanosis, less edema, infection disappearing.

8-19 — Gums bleed only when "brushed violently"; general appearance shows marked improvement.

9-2 — Progressive improvement, no bleeding, margins not entirely healed.

10-23 — Gums appear normal.

11-19 — Lowers show abnormal redness, marginal pallor.

12-16 — Increasing gingival irritation, slight infection at margin of left lower bicuspid.

2-25-43 — Gums normal.

3-24 — No change.

7-22 — No bleeding, some edema, poor color, marginal pallor, suggestion of recession (recurrent).

(Reports taking twenty-four vitamin pills since May).

prison subject. On his history sheet we found his report of regular daily consumption of one or two oranges for over 2 years.

Oral hygiene had been grossly neglected in a number of cases but at least half of the mouths examined had fair to excellent care. Bleeding of the gums on brushing was a common report. In fact small hemorrhages were visible on gums that had obviously been recently cleaned. Nine of the men were edentulous; six of this number were between 30 and 40 years of age. Many others had lost more than a third of their teeth. The reason for extraction was practically always recorded as "on account of pyorrhea". This complaint was so unusually frequent for men in their late 20's and early 30's that it appeared pertinent to inspect the details of the diet regularly served.

There was very little variation in the diet from week to week. Winter diet differed little from the summer diet. No fresh fruit or fruit juices and only small amounts of raw vegetables were listed. A typical week's diet is illustrated in table 1.

Furthermore, the preparation by steam cookery and the delay between preparation and consumption, which is unavoidable in the service of a large institution, make it improbable that more than a few milligrams of vitamin C are ingested per man through a day's food ration. Lack of teeth, of course, interfered with food intake, so that a "vicious circle" was established that interfered further with good nutrition in many of the inmates.

Gum inspection after a month's time on 25-mg. dose showed definite improvement of the oral tissues, even though little rise had occurred in the plasma vitamin C curve. The trend toward a normal pink color could be seen. Edema had decidedly subsided, particularly in mouths originally not too severely affected. With doses increased to 50 or 75 mg., as the rise in the plasma ascorbic acid became more pronounced, improvement in the appearance of the gums was more marked. Severe bleeding of the gums disappeared after 2 or 3 months if the blood ascorbic acid level was maintained in the neighborhood of 1.0 mg. %. In about the same period there was noted a tendency for pus pockets to clear, and if the normal blood level was maintained still longer some cases healed and new tissue appeared. Rise in the plasma values is reflected later in the improved appearance of affected gum tissues. The lag in signs of improvement was most pronounced in the individuals whose plasma values were most rapidly increased at the start by the larger doses. A similar lag in regression after the drop in plasma values occurred on withdrawal of supplements. Effect of withdrawal of the ascorbic acid supplement was noted in the plasma curves within

TABLE 1

Illustrated menus for the week ending June 5, 1943.

Sunday	Kix cereal Milk and sugar Stewed prunes Sweet rolls (2) Bread — butter Coffee	Baked swiss steak Brown gravy Mashed potatoes Creamed corn Rhubarb pie Bread — coffee	Sliced cold meat Fried potatoes Spice cake Raisin sauce Bread — syrup Coffee
Monday	Wheat cakes Syrup Bread Butter Coffee	Baked pork chop Brown gravy Oven brown potatoes String beans Apple cake Bread Coffee	Potato salad Sliced liver sausage Cup cakes Prune sauce Bread Syrup Coffee
Tuesday	Cream of wheat Milk and sugar Scrambled eggs Bread Butter Coffee	Baked Polish sausage Sauer kraut Steamed potatoes Rice pudding Bread Milk	Chili con carne Croutons Stewed apples Muffins Bread — syrup Coffee
Wednesday	Baked meat balls Fried potatoes Doughnuts Bread Syrup Coffee	Braised beef stew Mashed potatoes Beet greens Lemon pudding Bread Coffee	Wheat cakes Cold meats Stewed prunes Bread — butter Syrup Coffee
Thursday	Boiled oatmeal Milk and sugar Raisin sauce Filled rolls (2) Bread Butter Coffee	Breaded pork steak Lyonnaise potatoes Cold tomatoes Cream pie Bread Coffee	French toast Cold meat Fried potatoes Apple sauce Bread Syrup Coffee
Friday	Wheat cakes Syrup Bread Butter Coffee	Baked halibut Mashed potatoes Creamed peas Butterscotch pudding Bread Coffee	Sliced American cheese Cookies Rhubard sauce Bread Syrup Coffee
Saturday	Baked franks Hashed brown potatoes Hard rolls Bread Butter Coffee	Hamburger steak Oven brown potatoes Stewed carrots Orange pudding Bread Coffee	Baked pork and beans Doughnuts Cottage cheese Raisin sauce Bread — syrup Coffee

a week in our subjects. Plasma ascorbic acid dropped sharply below the "borderline" 0.8 level even if it had been carried for some time at a higher level. Four to 5 weeks after placebos were substituted for the supplements, plasma ascorbic acid was 0.2 mg. % or less for all subjects. The gums that had been normal in appearance again exhibited a slight puffiness and abnormal redness with occasional cyanosis.

Perhaps we did not give large enough doses to observe the striking results mentioned by Hanke ('30). But the types of lesions he describes were seen over and over. The most rapid recoveries were in the gums he calls the "soft and sometimes hypertrophied type" which are "very common" and show the smallest initial degree of tissue change. His "spongy gum" type represents a greater degree of involvement and is the gum that bleeds. This symptom when present did not disappear as rapidly as did edema and acute redness on treatment with supplements.

Comparing the notes and figures for July, 1943 with the original findings of May, 1942, we found that although the plasma ascorbic acid had dropped back to its original low level, the deterioration of the oral tissue, while pronounced, was by no means as severe in general, as the conditions originally observed. One suggestion we made had resulted in the serving of a slightly improved diet in the institution, which apparently somewhat more than compensated for the fact that less fruit is bought at present due to high prices. Beginning in the winter of 1942–43 canned tomatoes, served without reheating, have been offered in three or four meals a week. It was not formerly a popular food but is apparently growing in favor among the men, perhaps because of "propaganda", and may account for some of the delay in return of gum disease, even though it does not supply enough vitamin C to maintain good plasma levels. Five of the thirty-four subjects last examined had also been getting extra fruit, but in small quantities. Their plasma readings were between 0.2 and 0.4 %. Three others were taking vitamin pills, also in insufficient amounts. Their plasma readings were 0.59, 0.23 and 0 mg. %.

Consideration was given to the relationship between the severity of oral disease and age; also to the length of prison confinement (table 2). It was observed that the preponderance of severe disease is in the older group and also in those with the longest prison sentences.

Oral hygiene was noted for its possible connection with the prevalence of gum disorders. The first inspection showed that some men were regular in the care of their teeth. The prisoners are supplied with tooth

brushes and tooth powder for daily use. A full time dentist is employed by the prison. Many of them had made efforts to improve their gum conditions and were concerned over the necessity for frequent extractions. We therefore think that poor oral hygiene is not the major factor in the frequent severe gum disease seen in these prisoners. Prison diet seems to be the one constant factor which is responsible for high incidence of oral disease among the inmates. In every case where the diet was unsupplemented by extra fruit there was moderate to severe damage if the prison stay had been longer than 2 years. This

TABLE 2

Incidence of oral disease.

ORAL DISEASE	NO.	AGE GROUP		LENGTH OF PRISON CONFINEMENT, YEARS				REMARKS
		Over 30	Under 30	Over 10	5–10	2–5	1–2	
Severe	30	27	3	6	10	11	3	16 had no extra fruit
Moderate	14	6	8	..	3	4	7	2 had no extra fruit
Slight	27	11	16	..	3	12	12	3 had no extra fruit

was particularly significant in several of the younger subjects (less than 25 years old) whose teeth were excellent and mouths very clean but whose gums were reddened and edematous and bled at the least provocation.

DISCUSSION

As early as 1930 Hanke reported on the relationship between vitamin C deficiency and disease of the oral tissues. He described the conditions affecting the gums that were consistently observed in this study. His method of increasing the ascorbic acid intake was by feeding ½ to 1 pint of orange juice plus the juice of one half a lemon daily and including large quantities of raw cabbage or lettuce in the diet. We observed the same type of healing and gum improvement with the synthetic ascorbic acid which we gave in smaller doses over a longer period of time. Hanke's dosage probably furnished between 100 and 300 mg. of vitamin C from his daily prescribed fruit juices. Our primary interest was to find out how small a continuous dose would maintain the oral tissues in normal condition, since the larger doses may not now be obtainable, and should in any case, of course, not be wasted.

Tissue changes in vitamin C deficiency occur first in the supporting tissues of the teeth. Later changes affect the other supporting tissues of the body and are evidenced by slow healing of lesions and later by joint and bone changes. An adequate review of this subject has been presented by Wolbach and Bessey ('42). In our observations it was noted that among the large number of men with marked oral disease, accompanied by zero plasma ascorbic acid, there were several who participated in strenuous sports like wrestling and baseball. This was in addition to their regular work in the prison machine shop, twine plant, laundry, etc. All the men chosen were able to work steadily and were of the healthiest type of prisoner. The deficiency effects observed in oral tissues would appear to antedate practically any other detectable physical change from the normal.

Since the plasma is merely the vehicle by which the vitamin is distributed, the plasma values are the first to fall in deficiency states, but the last to return to normal when the tissues are resupplied. It has, therefore, been argued that a more quantitative index of severer degrees of deficiencies would be a measurement of the vitamin in blood cells, or whole blood that includes the cells. So far, only the plasma determination is technically practicable for survey studies like the present one. While it cannot be relied upon to differentiate among the severer degrees of tissue desaturation, we can certainly be safe in inferring from the very low plasma values found so consistently in our untreated subjects that supplies to the tissues were far from adequate. This is supported by the now widely accepted fact that in well nourished individuals plasma ascorbic acid concentrations are found nearly always above 0.7 or 0.8 mg. %, while under dietary conditions called optimal, and in the absence of illness or infection, levels in the plasma remain usually above 1.0 mg. %. The agreement between plasma ascorbic acid level and gum changes was so close throughout this study that it seems justifiable to use the plasma readings, or better, their response to added vitamin C as a criterion for determining the minimal needs.

In following out the separate curves for each of the men on the different supplements, it became apparent that ingestion of 50 mg. a day could not be called adequate. On the regular prison diet, entirely unsupplemented, we found certain controls whose plasma ascorbic acid stayed at zero the year round. Those controls who showed a slight seasonal rise were able to obtain some extra fruit at that time. A 3-year study of an institutional diet by Horwitt ('42) included plasma ascorbic acid studies on 380 "average patients" in a hospital for the insane. He

estimated that their daily diet included not more than 25 mg. daily. The lowest average figure for plasma ascorbic acid (0.2 mg. %) was found during the months of March to June. The highest average value occurred in October and November (0.59 mg. %) while the over all average was less than 0.4 mg. %. Barahal and Priestman ('42) reported 0.419 mg. % as the average plasma ascorbic acid in a group of forty-four mental patients whe were maintained on an unsupplemented hospital diet over a long period of time. They state that "a definite correlation has been found between the frequency of dental and gingival conditions in mental patients and their ascorbic acid levels".

A detailed study of the vitamin C requirements of six healthy female individuals was made by Storwick and Hauck ('42). They conclude that to maintain tissue saturation 65 to 150 mg. of ascorbic acid must be given in addition to approximately 10 mg. in the diet. They pointed out, as have others, wide individual variations. Fincke and Landquist ('42) set 0.8 mg. % as their standard for normal plasma ascorbic acid level. They found it required 0.8–1.2 mg. of ascorbic acid per kilo. body weight per day to maintain this level. This individual variation in vitamin C needs is becoming generally recognized. In such a group as we have studied, conditions outside the individual physiology were uncommonly standardized in comparison with those ordinarily obtainable in a similar-sized healthy group. Variations became apparent in the response of both blood levels and health of oral tissues to added amounts of vitamin C.

CONCLUSIONS

The probable minimal daily amount of vitamin C needed by healthy male adults lies in the neighborhood of 75 mg., as gauged by the response of the plasma ascorbic acid values and health of the gum tissues. Refractory individuals with low grade chronic infections such as nose and throat infections or deeply diseased gums appear to need at least 100 mg. daily to obtain a satisfactory response.

There appears to be a correlation between the duration of vitamin C deficiency and the severity of oral disease.

Ascorbic acid in institutional diets should be provided for by citrus and other fresh fruits, by increased amounts of tomatoes either fresh or canned (but not reheated), by supplements of synthetic ascorbic acid, or by a combination of these.

SUMMARY

The daily vitamin C requirements of seventy-one male prisoners were investigated over a period of 17 months.

Oral disease was prevalent among the younger as well as the older subjects.

The daily food lists revealed that no fresh fruit and very few fresh vegetables were included in the diet.

Laboratory results of plasma ascorbic acid determinations in untreated individuals showed that only a very few men, who were securing oranges or vitamin preparations for themselves, had plasma values over 0.6 mg. %; nearly 80% were under 0.2 mg. %, and twenty out of seventy-one showed no ascorbic acid in the plasma. Figures were very slightly higher in August and September than during the rest of the year.

Results of ascorbic acid supplements on plasma and gums were as follows: 25 mg. daily gave no significant elevation of the plasma values and only slight improvement in the gum disease; 50 mg. raised the plasma values noticeably, and definite improvement was noted in the gums, but this amount was not sufficient to maintain a plasma level of 0.8 mg. % during the winter and spring months. With a 75 or 100 mg. supplement the most satisfactory response of both plasma and gum healing was obtained. The 75 mg. also appeared sufficient for most of the men throughout the year. The few cases refractory to 75 mg. responded to an increase in dosage (100 mg.) within 4 to 5 weeks.

ACKNOWLEDGMENT

It is a pleasure to record our gratitude for the cooperation of a number of individuals. Mr. Albert Bailey, Secretary of the Wisconsin Board of Public Welfare, made arrangements authorizing the study in the State Institution. The enthusiastic cooperation of Mr. J. C. Burke, warden, shown by his presentation of the idea to the men and his readiness to advise with us about procedures, was an indispensable factor in the success of the study. We have enjoyed, on every occasion when we have requested it, the assistance of Dr. A. J. Hebenstreit, the physician to the prison; Dr. T. F. Meagher, the dentist; and Mr. I. C. Breitlow, the head nurse. The prison chef, Mr. Casper, has been very helpful. We are glad to acknowledge the sustained cooperation and dependability in reporting details of the participating inmates of the prison throughout the investigation.

The counsel and critical advice of Dr. C. A. Elvehjem and Dr. H. T. Parsons are sincerely appreciated.

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